Electronic Supplementary Information for:

Photodynamic ROS inducers delivered via electrostatic antibody targeted (ELART) vesicles incorporate into tumour cells and inhibit colony growth

Andreas Faust*[a], Andrew F. Berdel*[b], Evelyn Bruns*[b], Nicole Bäumer[b], Timo Krassuski[b], Lisa Wittmann[b], Linnea Franke[b], Iván Maisuls[c,d], Stephan Niland[e], Johannes A. Eble[e], Cristian A. Strassert[c,d], Georg Lenz[b], Wolfgang E. Berdel[b] , Sebastian Bäumer[b]

[a] *European Institute for Molecular Imaging, University of Münster, Waldeyerstr. 15, 48159, Münster, Germany. E-Mail: faustan@uni-muenster.de*
[b] *Department of Medicine A, Hematology/Oncology, University Hospital Münster, Albert-Schweitzer Campus 1, 48149 Muenster, Germany. E-Mail: baumers@uni-muenster.de*
[c] *Institut für Anorganische und Analytische Chemie, Universität Münster, Corrensstraße 28/30, 48149 Münster, Germany*
[d] *CiMIC, SoN, CeNTech, Universität Münster, Heisenbergstraße 11, 48149 Münster, Germany.*
[e] *Institute of Physiological Chemistry and Pathobiochemistry, University of Münster, Waldeyerstraße 15, 48149 Münster, Germany*
** These authors contributed equally to this work*

Author contributions

AF, NB, CS, GL, WEB and SB conceived and directed this study, designed and interpreted experiments, contributed scientific knowledge and obtained financial support. AFB, EB, TK, RM, LW, LF, SN and IM conducted, analysed and interpreted experiments. AF, AFB, NB and SB wrote the manuscript. All authors have read and agreed to the final version of the manuscript.

We thank Claudia Essmann and Renato Margeta for technical assistance.

Content

1. General information and materials (chemical synthesis)

Unless otherwise stated, all solvents and reagents were used as received from commercial suppliers without further purification. Reaction progress was monitored by TLC performed on aluminium plates coated with silica gel 60 F_{254} from Merck. Chromatograms were visualized by fluorescence quenching with UV light at λ=254 nm and by staining with iodine vapour or staining solutions. Following TLC staining solutions were used: 2 mL anisaldehyde and 8 mL conc. H_2SO_4 in 200 mL EtOH abs.; 8 g $Ce(SO_4)_2*2H_2O$ dissolved in 100 mL 15% aq. sol. H_2SO_4; 0.6 g ninhydrin dissolved in 200 mL abs. EtOH with 6 mL conc. AcOH.

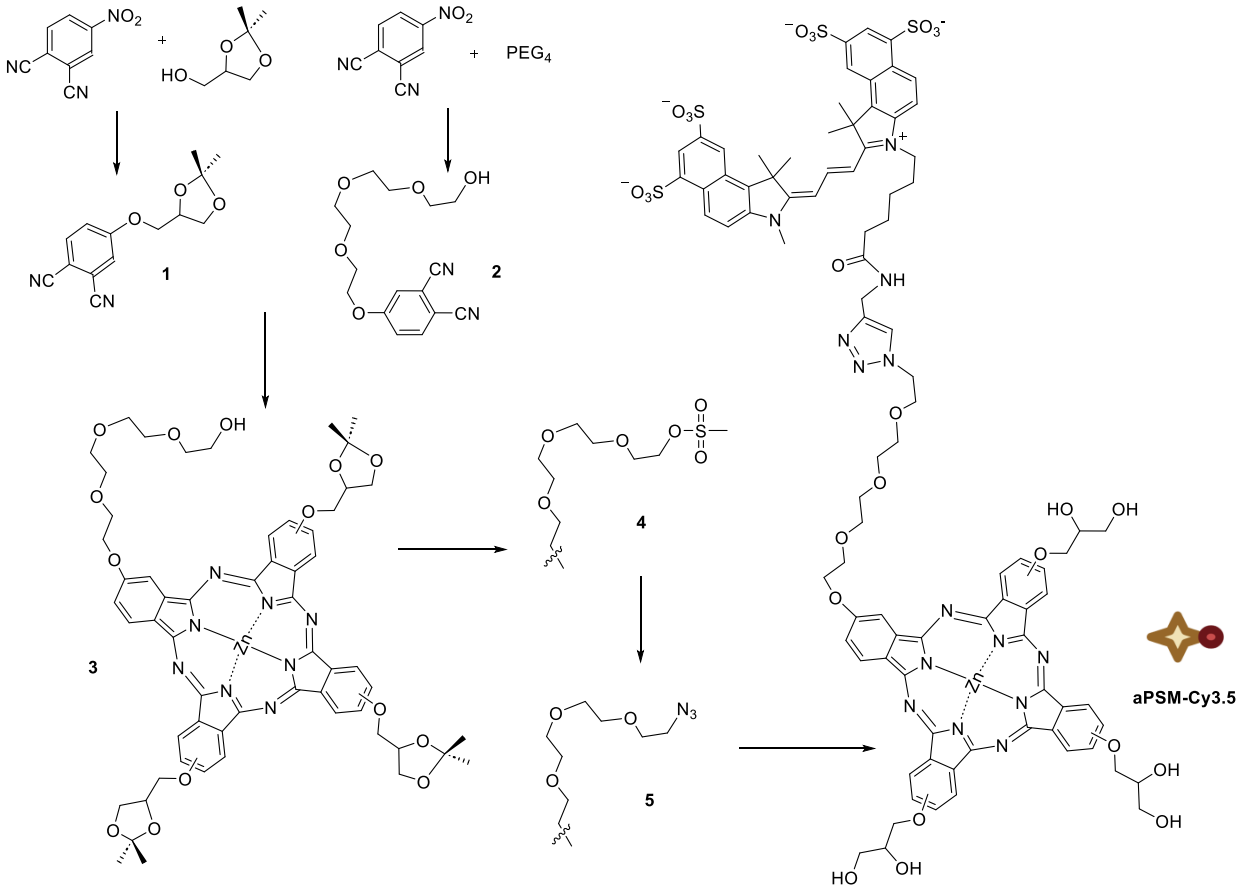
Column chromatography was performed manually on columns with ultrapure 60Å silica gel 60-200 µm from Acros or commercially available Waters SPA C-18 plus cartridges (purification of aPSM-Cy3.5).

All mass and NMR spectra were obtained from the Mass Spectrometry and NMR Departments of the Organic Chemistry Institute at the University of Münster. Mass spectra were obtained using Bruker MicrOTof or Autoflex Speed MALDI-TOF, Thermo Fisher Scientific Orbitrap LTQ XL or Orbitrap Velos Pro spectrometers. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using Bruker Neo 400 and DD2 600 spectrometers at 300K. Chemical shifts are reported in parts per million (ppm, δ), shifts are referenced to the solvent peak of $CDCl_3$ (^{1}H: δ = 7.26 ppm; ^{13}C: δ = 77.16 ppm) or DMSO-d_6 (^{1}H: δ = 2.50 ppm; ^{13}C: δ = 39.52 ppm). Coupling constants are given in Hz (J). ^{1}H and ^{13}C NMR splitting patterns are signed as singlet (s), doublet (d), triplet (t), quartet (q) as they appeared in the spectrum. Splitting patterns that could not be interpreted or visualized easily are signed as multiplet (m).

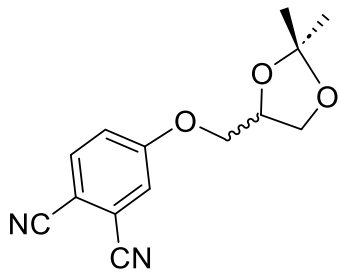
HPLC (quality control of aPSM-Cy3.5): Knauer HPLC system equipped with two Smartline 1000 pumps, UV detector Smartline 2500, and column Eurosphere II 100-10 C18, 250 x 4 mm with the following HPLC method: Mobile phase A = H_2O + 0.1% TFA; mobile phase B = ACN + 0.1% TFA; Flow = 3 mL / min; UV detector λ = 254 nm; Gradient 0 min – 5 % B, 2 min – 5 % B, 12 min – 95 % B, 15 min – 95 % B, 18 min – 5 % B, 20 min – 5 % B.

2. Synthesis of aPSM-Cy3.5

Scheme S1: Synthesis of **aPSM-Cy3.5**. In part (aPSM-Cy3.5 icon) *created in BioRender. Berdel, A. (2024) https://BioRender.com/k99v398*



***d,l*-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)phthalonitrile (1)**



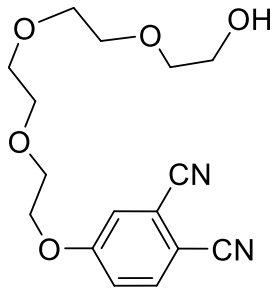
4-Nitrophthalonitrile (10 g, 57.8 mmol) and potassium carbonate (15.97 g, 115.6 mmol) were dissolved in dimethyl formamide (100ml) and heated to 50°C. While stirring *d,l*-1,2-isopropylidene-glycerole (10.8 ml, 86.7 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred 18h at 50°C. After complete conversion (control via TLC) the reaction mixture was poured into ice-cooled water (500ml), the precipitate was dissolved in CH_2Cl_2 and washed with water (3 x 150ml), brine (150ml), dried ($MgSO_4$). After evaporation of the solvent *in vacuo* the crude product was purified by column chromatography (cyclohexane : EtOAc 3:1) yielding **1** (11.3 g, 76%) as an off-white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.72 (d, 1H, J=8.8Hz, Ph*H*), 7.31 (d, 1H, J=2.6Hz, Ph*H*), 7.23 (dd, 1H, J=8.8, 2.6Hz, Ph*H*), 4.47-4.52 (m, 1H, C*H*), 4.18 (dd, 1H, J=8.6, 6.5Hz, CH_2), 4.13-4.09 (m, 2H, CH_2), 3.90 (dd, 1H, J=8.6, 5.6Hz, CH_2), 1.44 (s, 3H, CH_3), 1.39 (s, 3H, CH_3).

$^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ (ppm) = 161.8, 135.4, 119.8, 119.6, 117.7, 115.7, 115.3, 110.4, 108.8, 73.6, 69.8, 66.4, 26.9, 25.3.

The NMR data is consistent with the literature. [1]

4-[12-Hydroxy-(1,4,7,10-tetraoxadodecyl)] phthalonitrile (2)



4-Nitrophthalonitrile (5.6 g, 32.4 mmol) and potassium carbonate (8.79 g, 64.9 mmol) were dissolved in dimethyl formamide (100ml) and heated to 50°C. After tetraethylene glycol (33.7 ml, 195 mmol) was added dropwise over 30 minutes, the reaction mixture was stirred at 50°C for 18h. After complete conversion (TLC control) the reaction mixture was diluted with water (200 ml), extracted with toluene (3 x 100 ml) and the combined organic layers were dried ($MgSO_4$) and the solvent was removed in vacuo. The crude product was purified by column chromatography (EtOAc : methanol 98:2 -> 95:5) yielding **2** (1.83 g, 21%) as colourless oil.

^1H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.70 (d, 1H, J=8.8Hz, Ph*H*), 7.33 (d, 1H, J=2.6Hz, Ph*H*), 7.23 (dd, 1H, J=8.8, 2.6Hz, Ph*H*), 4.24-4.21 (m, 2H, OCH_2), 3.89-3.86 (m, 2H, OCH_2), 3.72-3.59 (m, 12H, OCH_2), 2.55-2.52 (m, 1H, O*H*).

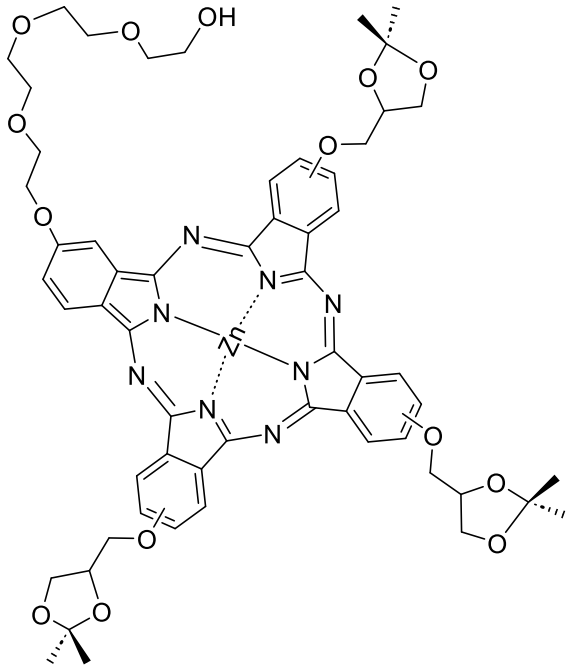
$^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ (ppm) = 162.1, 135.3, 120.0, 119.8, 117.5, 115.8, 115.4, 107.6, 72.6, 71.0, 70.8, 70.6, 70.4, 69.4, 68.7, 61.8.

The NMR data is consistent with the literature.[2]

[1] Y Zorlu, MA Ermeydan, F Dumoulin, V Ahsen, H Savoie and RW Boyle, *Photochem. & Photobiol. Sci.* 2009, **8**, 312-318.

[2] M Karabörk and S. Serin, *Synth. React. Inorg. Met.-Org. Chem.*, 2002, **32**, 1635-1647.

[2(3),9(10),16(17)-tri-(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy-23-(12-hydroxy-1,4,7,10-tetraoxadodecyl)]phthalocyaninato Zn(II) (3) ***mixture of multiple regioisomeres.***[3]



In a sealed tube under argon phthalonitrile **1** (200 mg, 0.77 mmol) and phthalonitrile **2** (25 mg, 0.077 mmol) were dissolved in ethanol (2 ml). $Zn(OAc)_2*2H_2O$ (68 mg, 0.308 mmol) and triethylamine (600 µl, 4.3mmol) were added via stirring and the tube was purged with argon, capped and heated to 120°C for 6h. During reaction a color change from light green to dark-green/black was observed, after cool down at RT the reaction mixture was poured dropwise into hot cyclohexane (60°C), after precipitation the cyclohexane phase was decanted and the precipitation was dissolved in 2ml dichloromethane and this washing procedure was repeated twice. The green precipitation was purified by column chromatography (CH_2Cl_2 : THF 3:1 -> 2:1 -> 1:1) yielding **3** (44 mg, 38 µmol, 49%) as deeply green solid.

^{1}H NMR (600MHz, d_6-DMSO): δ = 8.92-8.78, 8.50-8.34, 7.74-7.50, 7.35-7.19 (4m, 12H, Ph*H*), 4.78-4.09 (m, 18H, C*H, C*H_2), 3.85-3.38 (m, 14H, *C*H_2, O*H*), 1.62-1.59, 1.51-1.49, 1.37-1.31 (m, 18H, *C*H_3).

^{13}C{^{1}H} NMR (151MHz, d_6-DMSO): δ = 168.8, 163.5, 163.4, 161.8, 160.9, 159.9, 139.6, 135.2, 131.1, 130.8, 124.7, 124.5, 120.4, 117.6, 109.1, 109.1, 108.9, 108.3, 104.8, 74.1, 73.6, 73.5, 72.4, 72.3, 70.2, 70.0, 69.9, 69.8, 69.7, 69.6, 69.5, 69.4, 69.3, 68.7, 68.3, 66.0, 65.5, 65.4, 60.2, 48.6, 26.8, 26.8, 26.5, 25.6, 25.4. (overlapping and partially very weak signals).

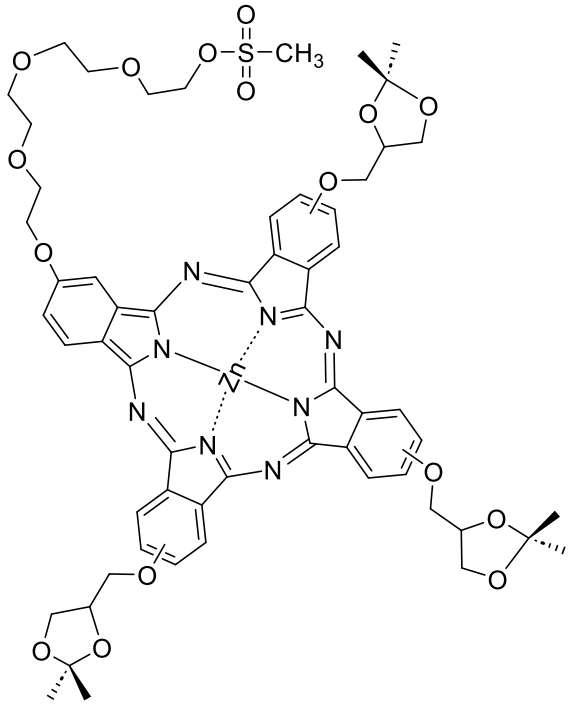
MALDI-ToF MS (DHB, EtOAc): mass calculated for $[M]^+$ ($C_{58}H_{62}N_8O_{14}Zn$), *radical cation*, required: *m/z* 1158.37, 1159.37, 1160.37, 1161.37, 1162.37, 1163.37, 1164.37, 1165.37; found: *m/z* 1158.41, 1159.42, 1160.42, 1161.43, 1162.42, 1163.43, 1164.43, 1165.43.

[2(3),9(10),16(17)-tri-(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy-23-(12-methylsulfonyloxy-1,4,7,10-tetraoxadodecyl)]phthalocyaninato Zn(II) (4) ***mixture of multiple regioisomeres.***[3]

In inert atmosphere (argon) alcohol **3** (44 mg, 38 µmol) was dissolved in dry dichloromethane (5 mL) and cooled down to 4°C. Dry triethylamine (25 µL, 0.19 mmol) was added and after two minutes methanesulfonyl chloride (12 µL, 0.15 mmol) was added dropwise and the reaction mixture was stirred for 1h. After TLC showed complete conversion, the solution was poured into water (10 mL) and washed with dichloromethane (3 x 10 mL), the combined organic layers

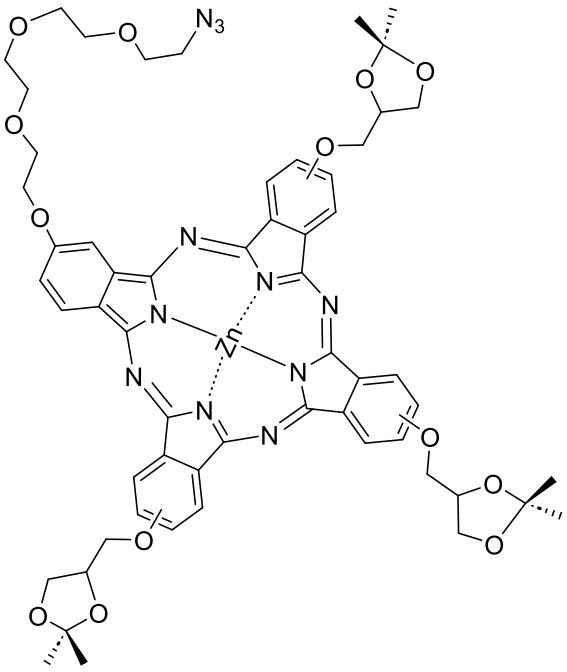
[3] Y Zorlu, F Dumoulin, D Bouchu, V Ahsen and D Lafont, *Tetrahedron Lett.* 2010, **51**, 6615-6618.

were dried ($MgSO_4$) and evaporated in vacuo yielding **4** as a green solid (40 mg, 32 µmol). After MS analysis **4** was used in the next step without further purification.



MALDI-ToF MS (DHB, $CHCl_3$): mass calculated for $[M]^+$ ($C_{59}H_{64}N_8O_{16}SZn$), *radical cation*, required: *m/z* 1236.34, 1237.35, 1238.34, 1239.34, 1240.34, 1241.34 1242.35, 1243.35; found: *m/z* 1236.28, 1237.29, 1238.28, 1239.29, 1240.29, 1241.29 1242.29, 1243.29.

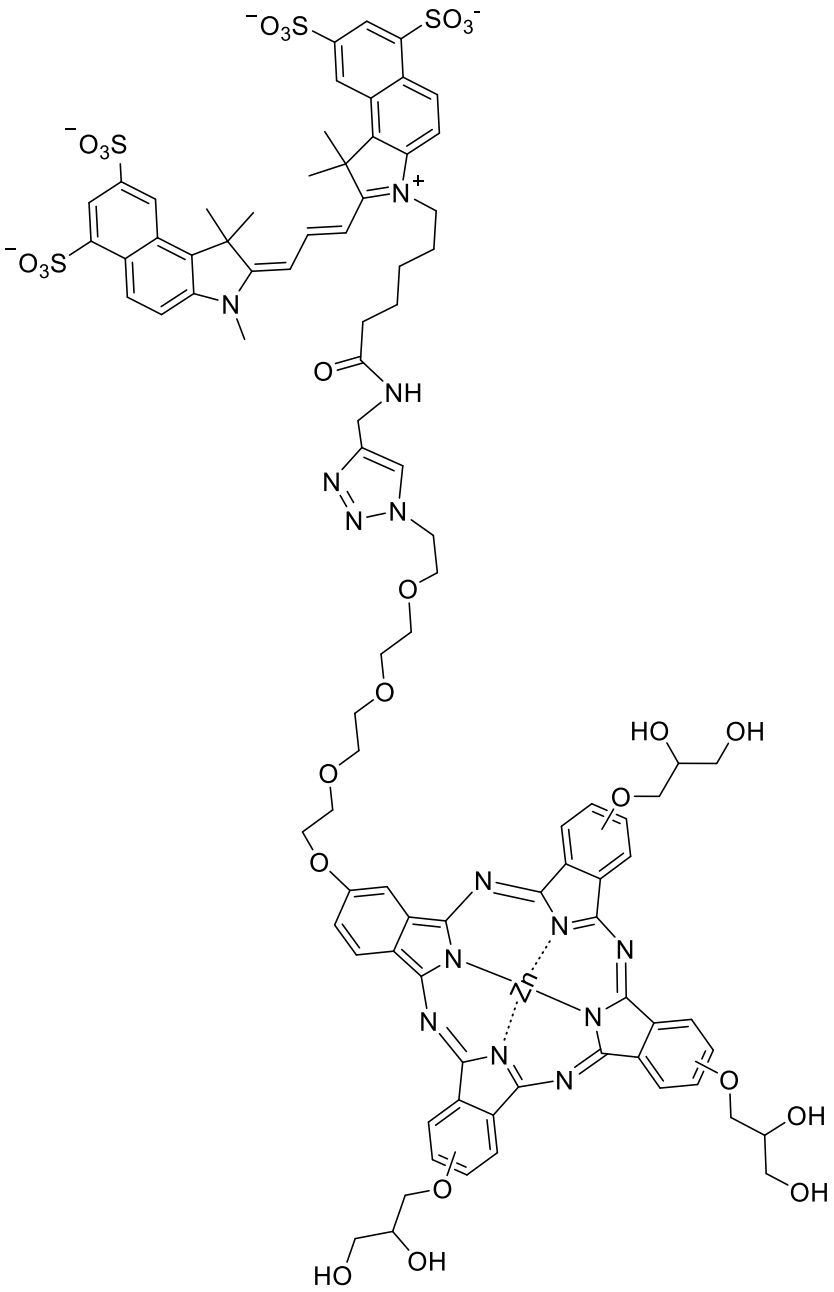
[2(3),9(10),16(17)-tri-(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy-23-(12-azido-1,4,7,10-tetraoxadodecyl)]phthalocyaninato Zn(II) (5) ***mixture of multiple regioisomeres.***[3]



Mesylate **4** (40 mg, 32 µmol) and sodium azide (8.4 mg, 0.13 mmol) were dissolved in dimethylformamide (2.5 mL) and stirred overnight at 100°C. After cooling to room temperature, the reaction mixture was poured into water (7.5 mL) and extracted with CH_2Cl_2 (3 x 7.5 mL) and the combined organic layers were dried ($MgSO_4$) and evaporated in vacuo. The resulting dark precipitate was co-evaporated subsequently with toluene, cyclohexane and EtOAc yielding **5** as dark green solid (30 mg, 26 µmol). After MS analysis **5** was used in the next step (partially) without further purification.

MALDI-ToF MS (DHB, $CHCl_3$): mass calculated for $[M]^+$ ($C_{58}H_{61}N_{11}O_{13}Zn$), *radical cation*, required: *m/z* 1183.37, 1184,38, 1185.37, 1186.37, 1187.37, 1188.37, 1189.38; found: *m/z* 1183.37, 1184,37, 1185.37, 1186.37, 1187.37, 1188.37, 1189.37.

1,1-dimethyl-3-(6-oxo-6-(((1-(2-(2-(2-(2-((9(10),16(17),23(24)-tris(2,3-dihydroxypropoxy)phthalo-cyanine-2-yl)oxy)ethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)amino)hexyl)-2-((1*E*,3*E*)-3-(1,1,3-trimethyl-6,8-disulfonato-1,3-dihydro-2H-benzo[e]indol-2-ylidene)prop-1-en-1-yl)-1H-benzo[e]indol-3-ium-6,8-disulfonato Zn(II) (aPSM-Cy3.5)



In a vial protected from light, azide **5** (6 mg, 5.1 µmol) and sulfo-cyanine-3.5-alkyne (Cat. D23B0, Lumiprobe Life Science Solutions, Hannover, Germany) (5 mg, 5.1 µmol) were dissolved in dimethyl formamide (0.5 mL). To this solution $CuSO_4$ * 5 H_2O (1.25 mg, 5 µmol, dissolved in 50 µL water) and Na-ascorbate (1.19 mg, 6 µmol, dissolved in 50 µL water) were given at once and the reaction mixture was stirred overnight at rt. The solvent was evaporated in vacuo and the crude product was subsequently co-evaporated with toluene and CH_2Cl_2, dissolved in water (20 mL) and washed with CH_2Cl_2 until the CH_2Cl_2-Phase was colourless. The crude conjugate in the aqueous phase was further purified by column chromatography:
5 mL portions were given on two connected and preconditioned (3 x 10 mL ACN, 3x 10 mL water) Waters SPA C18 plus cartridges:
Side-products were eluted with
5 x 10 mL H_2O (dark blue – light pink)
5 x 10 mL H_2O:methanol 9:1 (pink – light pink)
3 x 10 mL H_2O:methanol 8:2 (light pink – light purple)
Crude product (partially deprotected)[4] *was eluted with*
2 x 10 mL H_2O:methanol 6:4
2 x 10 mL H_2O:methanol 4:6
2 x 10 mL H_2O:methanol 2:8 (purple – light purple – clear).
All purple fractions were combined and evaporated to dryness.

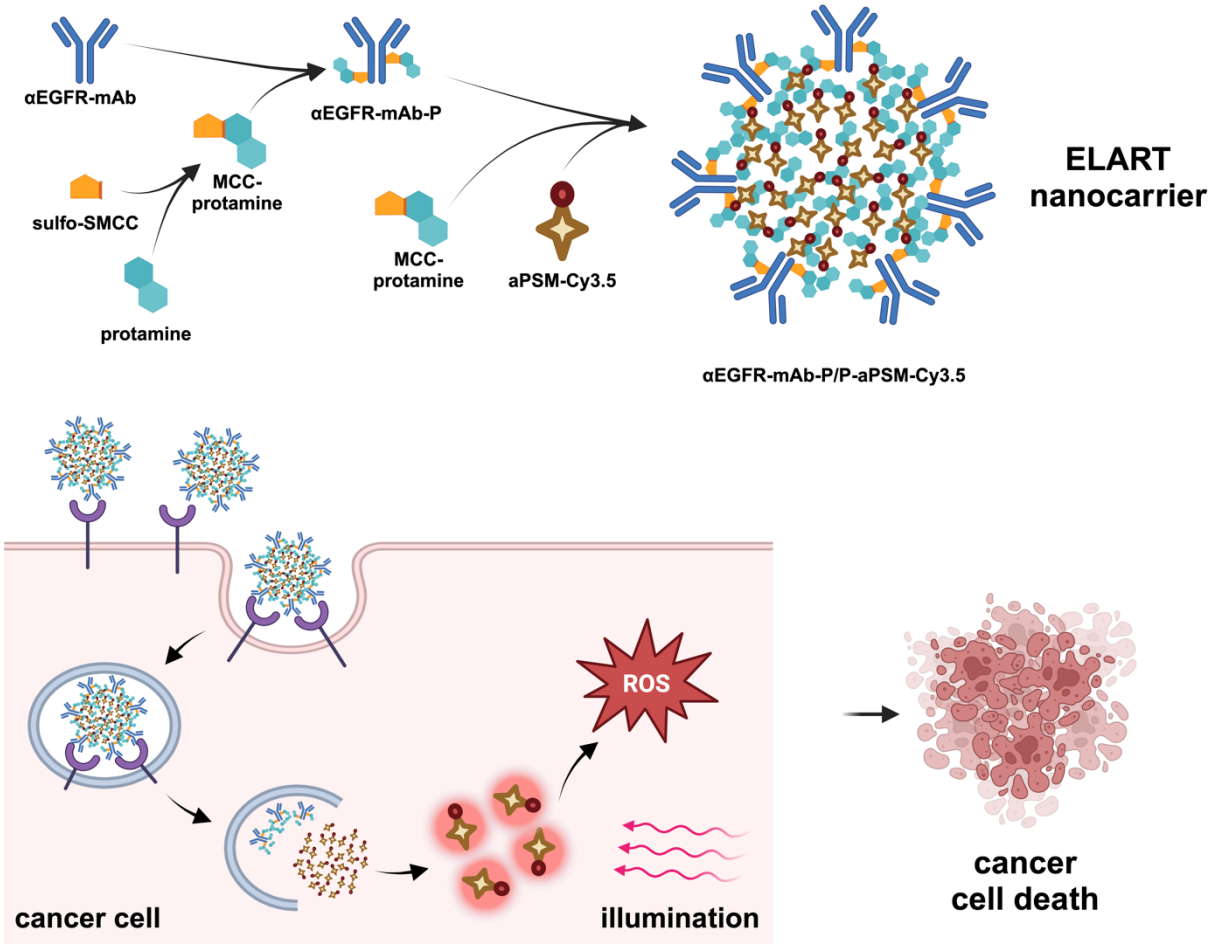
For complete deprotection in a light protected vial the crude product was dissolved in AcOH (300µl) and heated to 70°C over 1h. After cooling to room temperature, the reaction mixture was neutralized with saturated sodium bicarbonate and was purified by chromatography using a preconditioned (3 x 10 ml methanol, 3x 10 ml Water) SPA C18 plus short cartridge:
2 x 10 mL H_2O
2 x 10 mL H_2O:methanol 9:1
2 x 10 mL H_2O:methanol 8:2
2 x 10 mL H_2O:methanol 7:3
60 mL methanol (purple)

All purple fractions were lyophilized yielding 3.9 mg (2.0 µmol, 26%, over three steps) of **6** (**aPSM-Cy3.5**) as dark purple solid.

HRMS (ESI-): exact mass calculated for $[M]^{3-}$ ($C_{90}H_{89}N_{14}O_{26}S_4Zn$)
required *m/z* = 657.80877; 658.14310, 658.47570, 658.80927, 659.14201, 659.47557
found *m/z* = 657.80820, 658.14228, 658.47438, 658.80806, 659.14060, 659.47437.

[4] pH value of ~ 3 of the reaction mixture leads to partial deprotection of the acetonides

3. Preparation of the ELART nanocarrier αEGFR-mAb-P/P-aPSM-Cy3.5



Scheme S2: Preparation (upper) and mode of action (lower) of the ELART nanocarrier αEGFR-mAb-P/P-aPSM-Cy3.5. Created in BioRender. Berdel, A. (2024) https://BioRender.com/k99v398

Conjugation of sulfo-SMCC to protamine-sulfate and αEGFR monoclonal antibody cetuximab to sulfo-SMCC-protamine-sulfate

Conjugations of sulfo-SMCC to protamine and monoclonal antibody were performed as previously described.[5,6,7,8,9] In short, protamine sulfate (20 mg/dl ddH_2O; Cat. No. 539122, Calbiochem) was amino-terminally coupled to sulfo-SMCC (4.36 mg/ml in ddH_2O; Pierce No. 22622, Rockford, IL, USA) at a molar ratio of 1:5 in ddH_2O (pH adjusted to 6.0–7.0 with 0.1 M

[5] N. Bäumer, N. Appel, L. Terheyden, F. Buchholz, C. Rossig, C. Müller-Tidow, W. E. Berdel and S. Bäumer, *Nat Protoc*, 2016, **11**, 22-36..
[6] N. Bäumer, J. Rehkämper, N. Appel, L. Terheyden, W. Hartmann, E. Wardelmann, F. Buchholz, C. Muller-Tidow, W. E. Berdel and S. Bäumer, *PLoS One*, 2018, **13**, e0200163.
[7] N. Bäumer, A. Scheller, L. Wittmann, A. Faust, M. Apel, S. C. Nimmagadda, C. Geyer, K. Grunert, N. Kellmann, M. Peipp, S. Kailayangiri, M. E. Gutierrez Suburu, C. A. Strassert, M. Schenk, L. Greune, C. Rüter, P. Dersch, W. Hartmann, C. Rossig, D. Neri, C. Muller-Tidow, C. Schwoppe, C. Schliemann, C. Khandanpour, G. Lenz, W. E. Berdel and S. Bäumer, *J Hematol Oncol*, 2022, **15**, 171.
[8] S. Bäumer, N. Bäumer, N. Appel, L. Terheyden, J. Fremerey, S. Schelhaas, E. Wardelmann, F. Buchholz, W. E. Berdel and C. Muller-Tidow, *Clin Cancer Res*, 2015, **21**, 1383-1394.
[9] A. Faust, N. Bäumer, A. Schlütermann, M. Becht, L. Greune, C. Geyer, C. Ruter, R. Margeta, L. Wittmann, P. Dersch, G. Lenz, W. E. Berdel and S. Bäumer, *Angew Chem Int Ed Engl*, 2022, **61**, e202109769.

carbonate buffer (pH 8.3)) and incubated for 1 h at 37 °C, purified by gel filtration chromatography in Zeba spin desalting columns (Pierce No. 89891). Resulting protamine-sulfo-SMCC was coupled to cysteine residues of the αEGFR monoclonal antibody cetuximab (αEGFR-mAb) in a 32:1 molar ratio overnight at 4 °C. Coupling efficiency was analysed by SDS-PAGE and Coomassie staining as previously described.[5,6,7,8,9]

Preparation of ELART vesicles and formation control with bandshift assays

Ability of αEGFR-mAb-P/P to bind and carry aPSM-Cy3.5 and load estimation was performed with bandshift assays as previously described.[5,6,7,8,9] In brief, a constant amount of aPSM-Cy3.5 (1 µl = 500 pmol) and an increasing amount of αEGFR-mAb-P/P were mixed and incubated for 1 h at room temperature to determine the saturation of the antibody. Agarose gel electrophoresis was performed with 1.5 % (w/v) agarose gel with RedSafe in 1x TAE running buffer and monitored with UV transillumination imaging. Free compound was complexed by αEGFR-mAb-P/P to saturation of the compound loading capacity. This resulted in accumulation of non-migratory αEGFR-mAb-P/P bound to the compound in the gel pocket. The molecular ratio of appearance of free compound monomers depicts the maximum compound load capacity of the αEGFR-mAb-P/P preparation.

Analysis of nanocarrier formation by fluorescence microscopy

Nanocarrier assembly was analysed as previously described.[9] For cell-free nanoparticle-self-assembly studies, preformed αEGFR-mAb-P/P-aPSM-Cy3.5 nanocarriers were applied to glass slides overnight (o/n) to settle the nanoparticles by gravity and adhesion to the glass surface, washed with PBS, fixed with 4% PFA and mounted in DAKO mounting medium for microscopical analysis.

4. Photophysical characterisation

UV-visible absorption spectra were measured using a Shimadzu UV-VIS spectrophotometer (UV-3600i) equipped with a 50-W halogen lamp, a deuterium lamp, photomultiplier tube (UV/VIS), InGaAs photodiode (NIR) detectors, a high-performance blazed holographic grating type double monochromator, a pre-monochromator (concave grating spectrometer), and a main-monochromator (Czerny-Turner mount with aberration correction). The spectral bandwidth is 0.1 nm in the range of 185 nm to 3300 nm, and data were collected employing LabSolutions UV-Vis software.

Steady-state emission spectra was recorded on a FluoTime 300 spectrometer from PicoQuant equipped with a 300 W ozone-free Xe lamp (200-1100 nm), a 10 W Xe flash-lamp (200-1100 nm, pulse width ca. 1 µs) with repetition rates of 1 – 300 Hz, a double-grating excitation monochromator (Czerny-Turner type, grating with 1200 lines/mm, blaze wavelength: 300 nm), diode lasers (pulse width < 20 ps) operated by a computer-controlled laser driver PDL-820 "Sepia II" (repetition rate up to 80 MHz, burst mode for slow and weak decays), two emission monochromators (Czerny-Turner, selectable between double-grating blazed at 500 nm with 2.7 nm/mm dispersion and 1200 lines/mm, or single-grating blazed at 1250 nm with 5.4 nm/mm dispersion and 600 lines/mm) with adjustable slit width between 25 µm and 7 mm, Glan-Thompson polarizers for excitation (after the Xe-lamps) and emission (after the sample). Different sample holders (Peltier-cooled mounting unit ranging from -15 to 110 °C or an adjustable front-face sample holder), along with two detectors (namely a PMA Hybrid-07 from PicoQuant with transit time spread FWHM < 50 ps, 220 – 850 nm, or a H10330C-45-C3 NIR detector with transit time spread FWHM 0.4 ns, 950-1400 nm from Hamamatsu) were used. Steady-state spectra were recorded in TCSPC mode by a PicoHarp 300 (minimum base resolution 4 ps). Emission spectra were corrected for source intensity (lamp and grating) by standard correction curves. All solvents used were of spectrometric grade (Uvasol®, Merck).

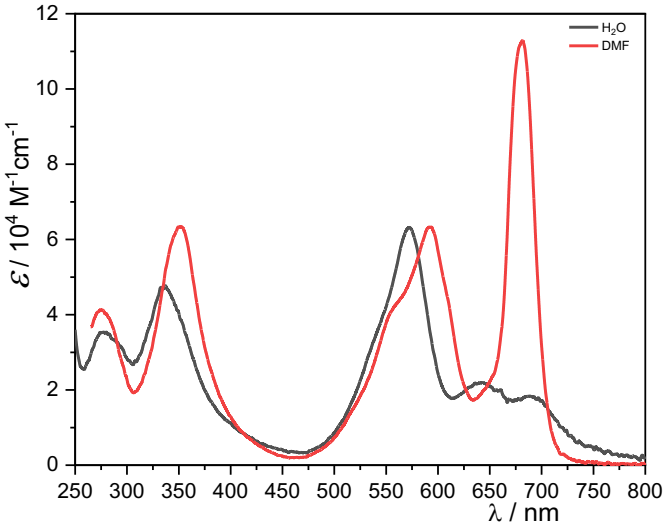


Figure S1: UV-vis absorption spectra (molar absorption coefficient as a function of wavelength) of **aPSM-Cy3.5** in H_2O (black) and DMF (red). Validity range: $c = 10^{-6} – 10^{-4}$ M at 298 K.

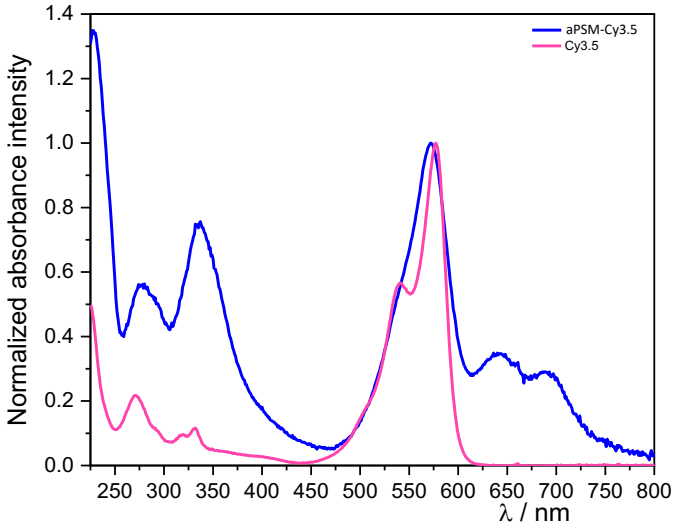


Figure S2: Normalized UV-vis absorption spectra of **aPSM-Cy3.5** (blue) and Cy3.5 (pink) in H_2O at 298 K, referenced to the λ_{max} of Cy3.5).

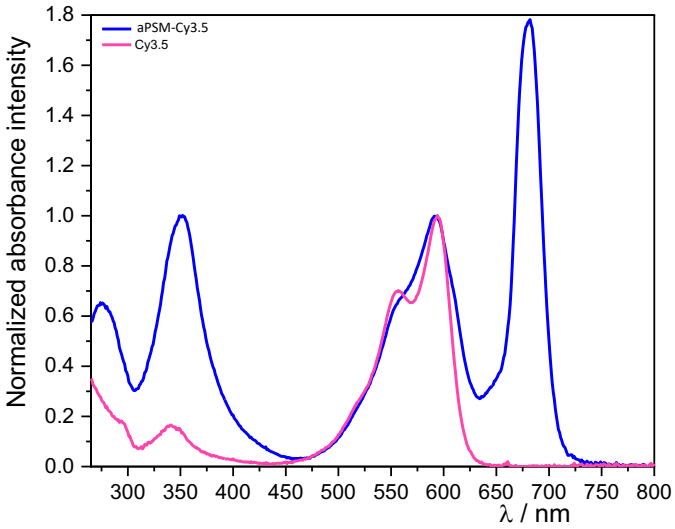


Figure S3: Normalized UV-vis absorption spectra of **aPSM-Cy3.5** (blue) and Cy3.5 (pink) in DMF at 298 K, referenced to the λ_{max} of Cy3.5).

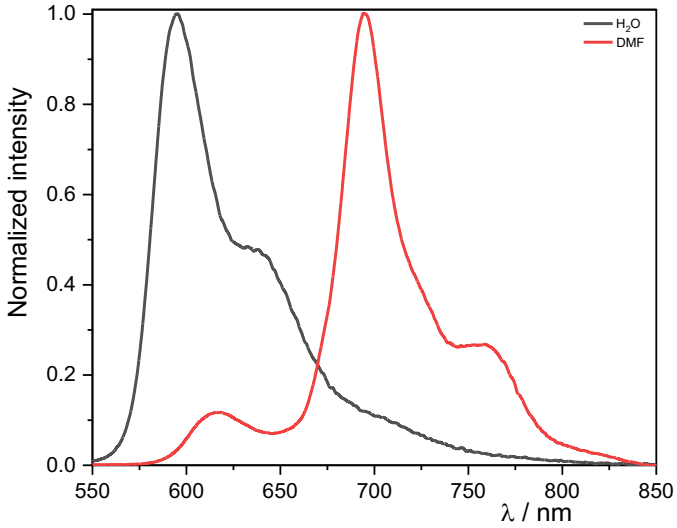


Figure S4: Normalized emission spectra (λ_{ex} = 500 nm, c = 10^{-5} M) of **aPSM-Cy3.5** in H_2O (black) and DMF (red) at 298 K.

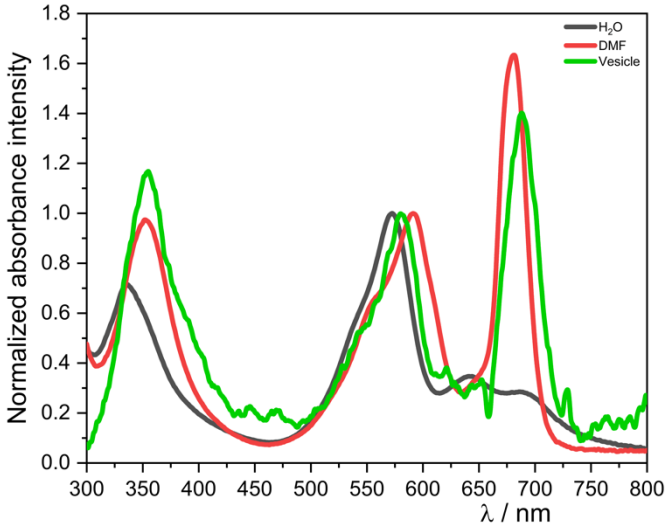


Figure S5: Normalized UV-vis absorption spectra of a suspension of αEGFR-mAb-P/P-aPSM-Cy3.5 nanocarriers ("vesicle") in H_2O (green), referenced to the λ_{max} of Cy3.5. The spectra of **aPSM-Cy3.5** in H_2O and DMF are included for comparison (black dotted line and red dotted line, respectively) at 298 K.

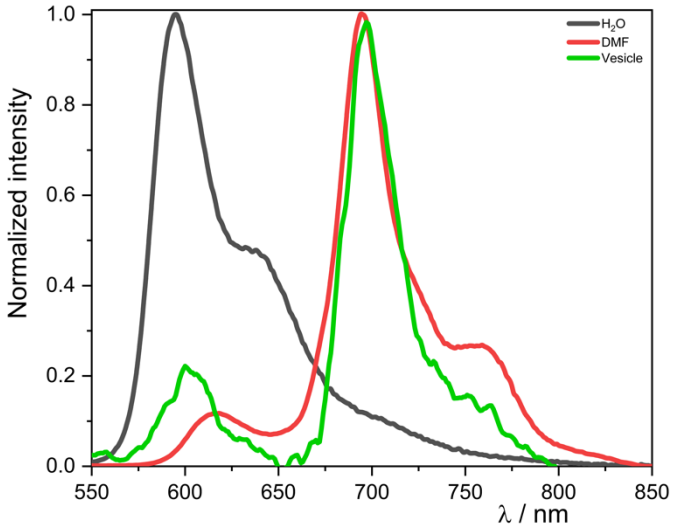


Figure S6: Normalized emission spectra (λ_{ex} = 500 nm) of a suspension of αEGFR-mAb-P/P-aPSM-Cy3.5 nanocarriers ("vesicle") in H_2O (green). The spectra of **aPSM-Cy3.5** in H_2O and DMF are included for comparison (black dotted line and red dotted line, respectively) at 298 K.

5. Cell culture and *in vitro* evaluation

Human EGFR-positive non-small cell lung cancer cell lines A549 and SK-LU1 and pancreatic cancer cell lines MiaPaCa2 and Panc1 were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin (P/S). SK-LU1 medium was additionally supplemented with 1% sodium pyruvate (SP). Colorectal carcinoma cell lines DLD1 and SW480 were cultured in RPMI supplemented with 10 % FBS and 1% P/S. Cells were incubated at 37°C with 5% CO_2 and high humidity. Cell lines were routinely tested for mycoplasma with PCR. Nanocarrier treatment was performed as detailed in the following method sections as well as previously described.[5,6,7,8,9]

Detection of internalized vesicles in cells and vesicular nanocarriers by fluorescence microscopy

Internalization of nanocarriers was analysed as previously described.[9] For detection of internalized vesicles in cells, 60 nM αEGFR-mAb-P/P complex was coupled to 1800 nM aPSM-Cy3.5 for 2 h at RT. 5 x 10^4 cells were seeded and treated with these nanocarriers overnight at 37 °C and 5% CO_2. Subsequently, cells were washed with cold PBS, transferred to adhesion slides bordered with hydrophobic immunohistochemical pen and kept on ice, incubated for 20 min to adhere to surface, fixed with ice-cold 4% paraformaldehyde (PFA), stained with Hoechst33342, mounted with DAKO fluorescent mounting medium (lot. no. 10121691, DAKO, North America), covered with cover slips and photographed on a Nikon Eclipse 50i upright microscope.

Laser Scanning Microscopy (LSM)

For detection of internalized vesicles in cells, 180 nM αEGFR-mAb-P/P complex was coupled to 5400 nM aPSM-Cy3.5 for 1 h at RT. A549 and Panc-1 cells were seeded in ibidi treat 8 well slides (cat.no: 80826) at a density of 3.5x10^4 cells per well in 300 µL medium (DMEM, 10% FCS, 1% P/S). 90 minutes post-seeding cells were treated with these nanocarriers and incubated at 37°C and 5% CO_2 overnight. The next day cells were successively stained with 150 nM LysoTracker® Green DND-26 (Molecular Probes L7526) for 1 hour, 200 nM MitoView Fix 640 (Biotium #70082) for 20 minutes and 1 µg/mL Hoechst 33342 for 10 minutes in medium at 37°C and 5% CO_2. For imaging, the staining medium was replaced and cells were then imaged by using a Zeiss LSM 800 with a Plan-Apochromat 40x/1.4 Oil DIC (UV) VIS-IR M27 objective and lasers with a wavelength of 405 nm, 488 nm, 561 nm and 640 nm.

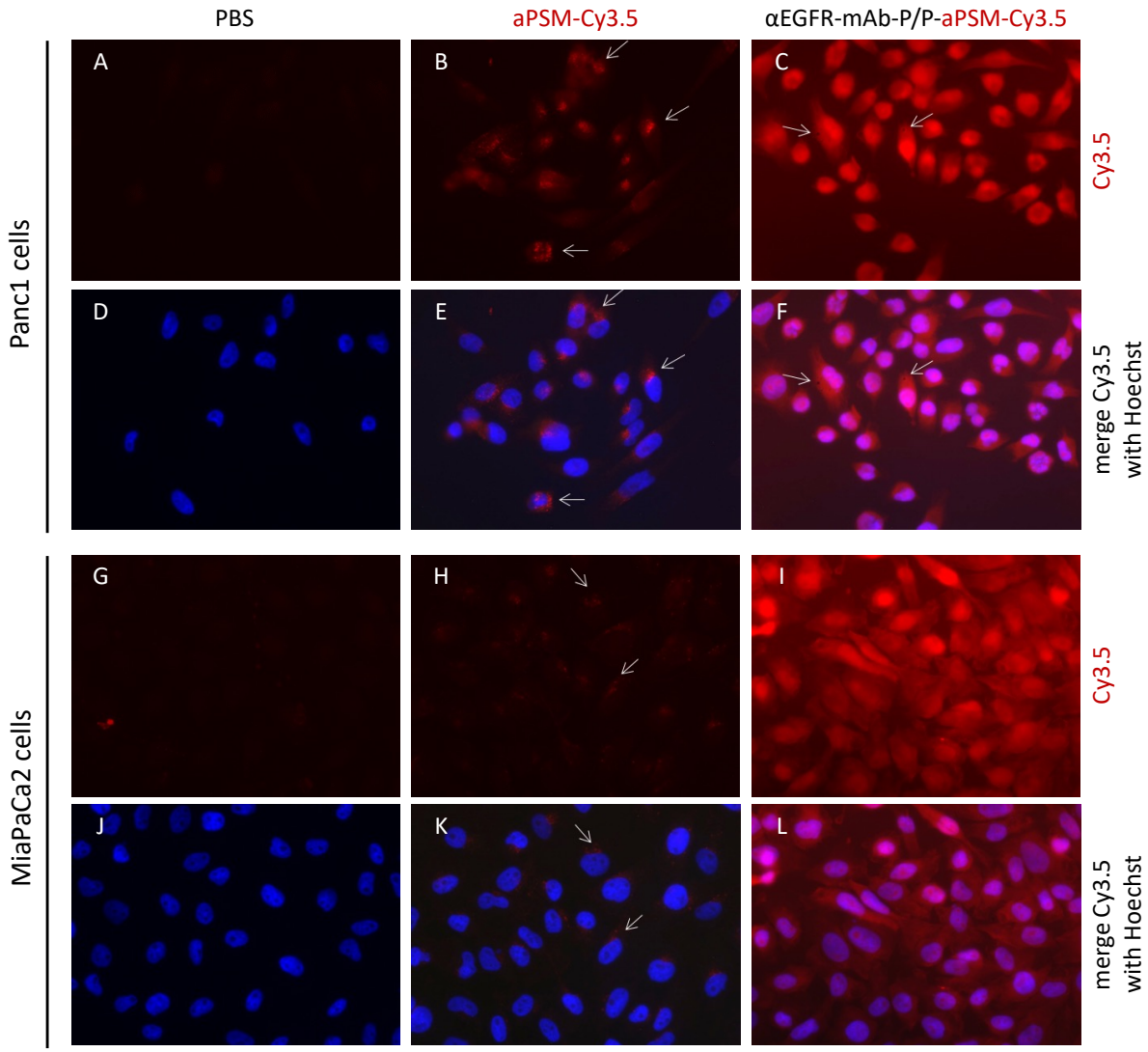


Figure S7: Fluorescence microscopy of Panc1 and MiaPaCa2 PC cells cells pre-treated with PBS-control (A, D, G, J) and analysis of aPSM-Cy3.5 (1800 nM) internalization (B, E, H, K), internalization mediated by αEGFR-mAb-P/P (60 nM) complexed with aPSM-Cy3.5 (1800 nM) (C, F, I, L). Red fluorescence displays aPSM-Cy3.5. Nanoparticle formation was performed for 1 h and a subsequent internalization overnight. A-C, G-I: Cy3.5 channel, D-F, J-L: Merged Hoechst and Cy3.5.

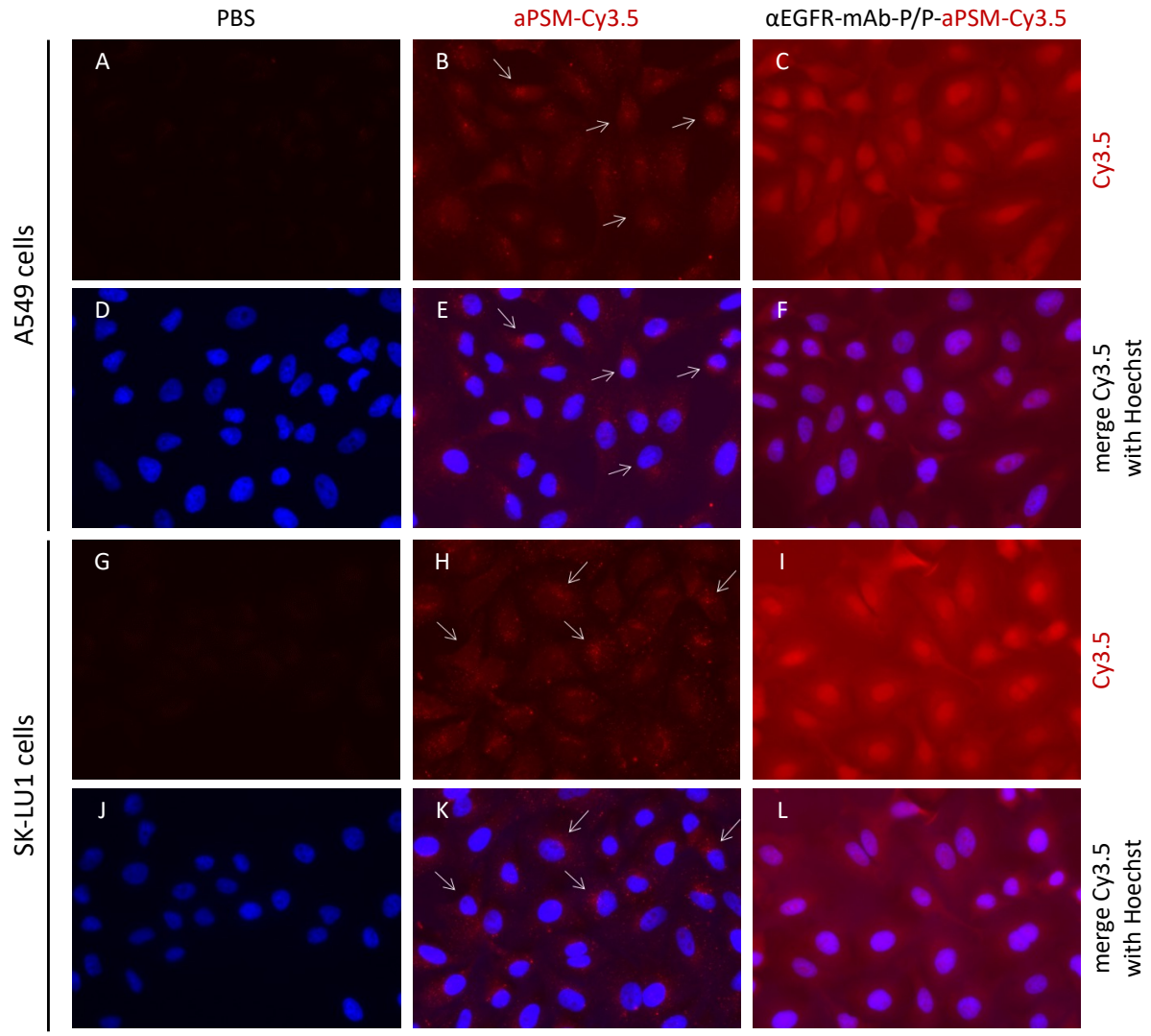


Figure S8: Fluorescence microscopy of A549 and SK-LU1 NSCLC cells pre-treated with PBS-control (A, D, G, J) and analysis of aPSM-Cy3.5 (1800 nM) internalization (B, E, H, K), internalization mediated by αEGFR-mAb-P/P (60 nM) complexed with aPSM-Cy3.5 (1800 nM) (C, F, I, L). Red fluorescence displays aPSM-Cy3.5. Nanoparticle formation was performed for 1 h and a subsequent internalization overnight. A-C, G-I: Cy3.5 channel, D-F, J-L: Merged Hoechst and Cy3.5.

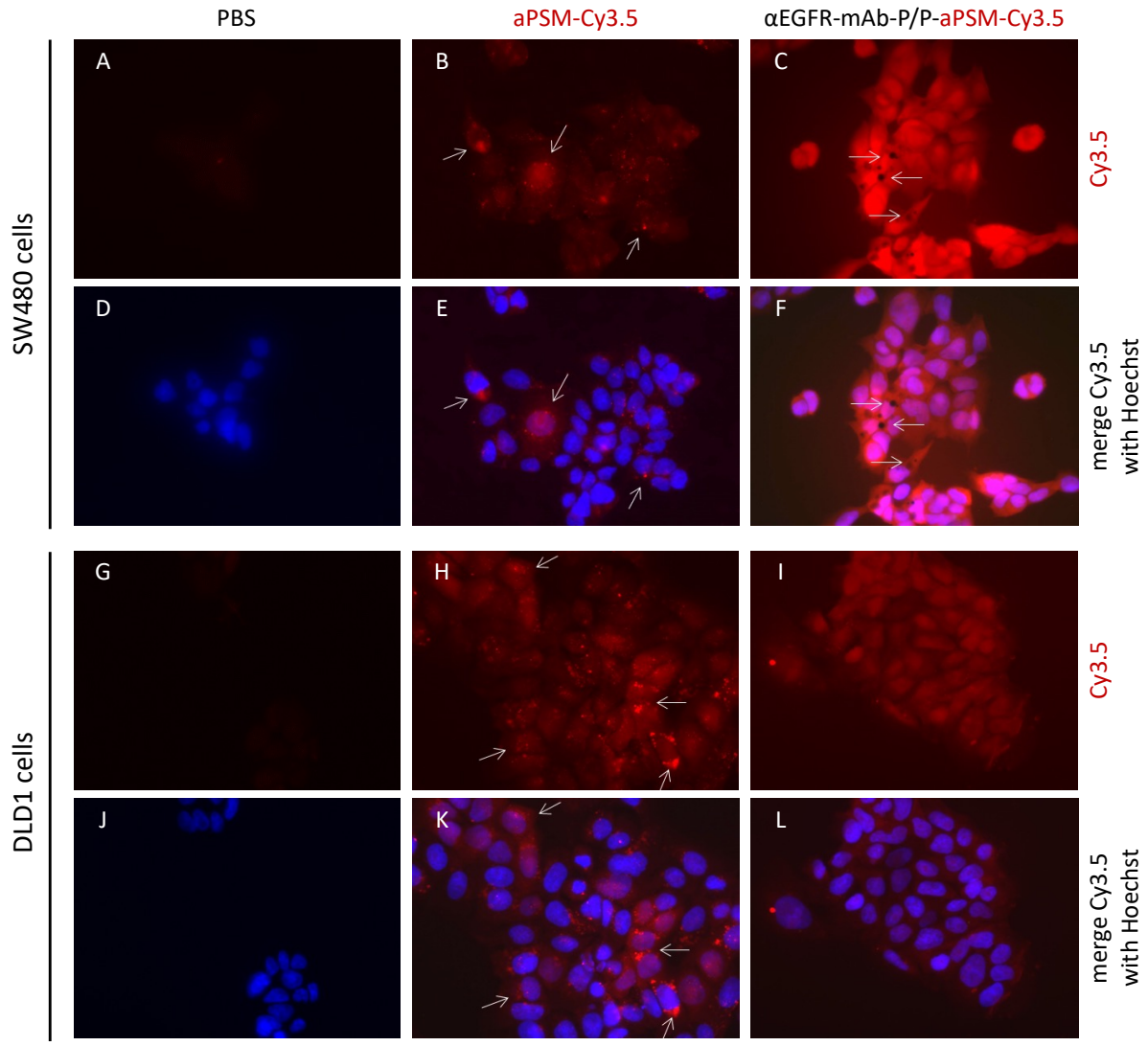


Figure S9: Fluorescence microscopy of SW480 and DLD1 CRC cells pre-treated with PBS-control (A, D, G, J) and analysis of aPSM-Cy3.5 (1800 nM) internalization (B, E, H, K), internalization mediated by αEGFR-mAb-P/P (60 nM) complexed with aPSM-Cy3.5 (1800 nM) (C, F, I, L). Red fluorescence displays aPSM-Cy3.5. Nanoparticle formation was performed for 1 h and a subsequent internalization overnight. A-C, G-I: Cy3.5 channel, D-F, J-L: Merged Hoechst and Cy3.5.

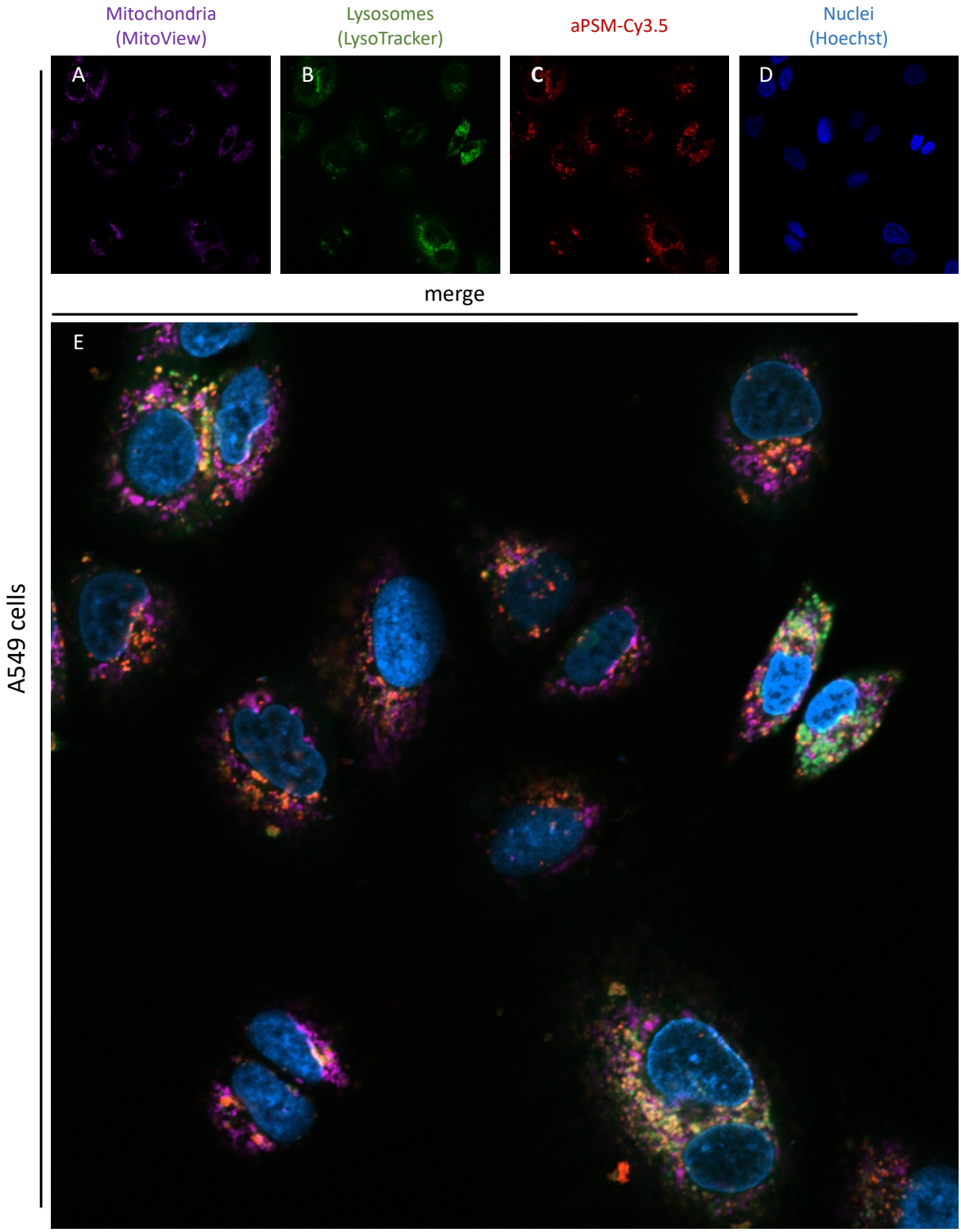


Figure S10: Laser scanning microscopy (LSM) analysis of internalization. A549 cells pre-treated with αEGFR-mAb-P/P (180 nM) complexed with aPSM-Cy3.5 (5400 nM). Nanoparticle formation was performed for 1 h and a subsequent internalization overnight. **A:** Mitochondria stained with MitoView Fix 640 are shown in purple. **B:** Lysosomes stained with LysoTracker Green DND-25 are shown in green. **C:** aPSM-Cy3.5 was detected by red fluorescence. **D:** Blue fluorescence displays nuclei stained with Hoechst 33342. E: Merged channels of A-D displaying subcellular localization of ELART Nanocarriers.

Flow cytometric analysis of internalization

For flow cytometric analysis of dose-dependent internalization. Nanocarriers were complexed with αEGFR-mAb-P/P coupled to aPSM-Cy3.5 at ratios of 30 nM:900 nM, 60 nM:1800 nM, 120 nM:3600 nM, 180 nM:5400 nM and 240 nM:7200 nM for 1 h at RT. Biological triplicates of Panc-1 cancer cell line were seeded in a 24 well plate at $1x10^6$ cells per well (in 600 µL medium) and treated with these nanocarriers at 37°C and 5% CO_2 for 2 h. After incubation the supernatant was discarded, cells were washed with PBS, detached by trypsination and the mean Cy3.5 signal of the healthy cell population was analyzed by flow cytometry with a FACSymphony A1 (BD Biosciences).

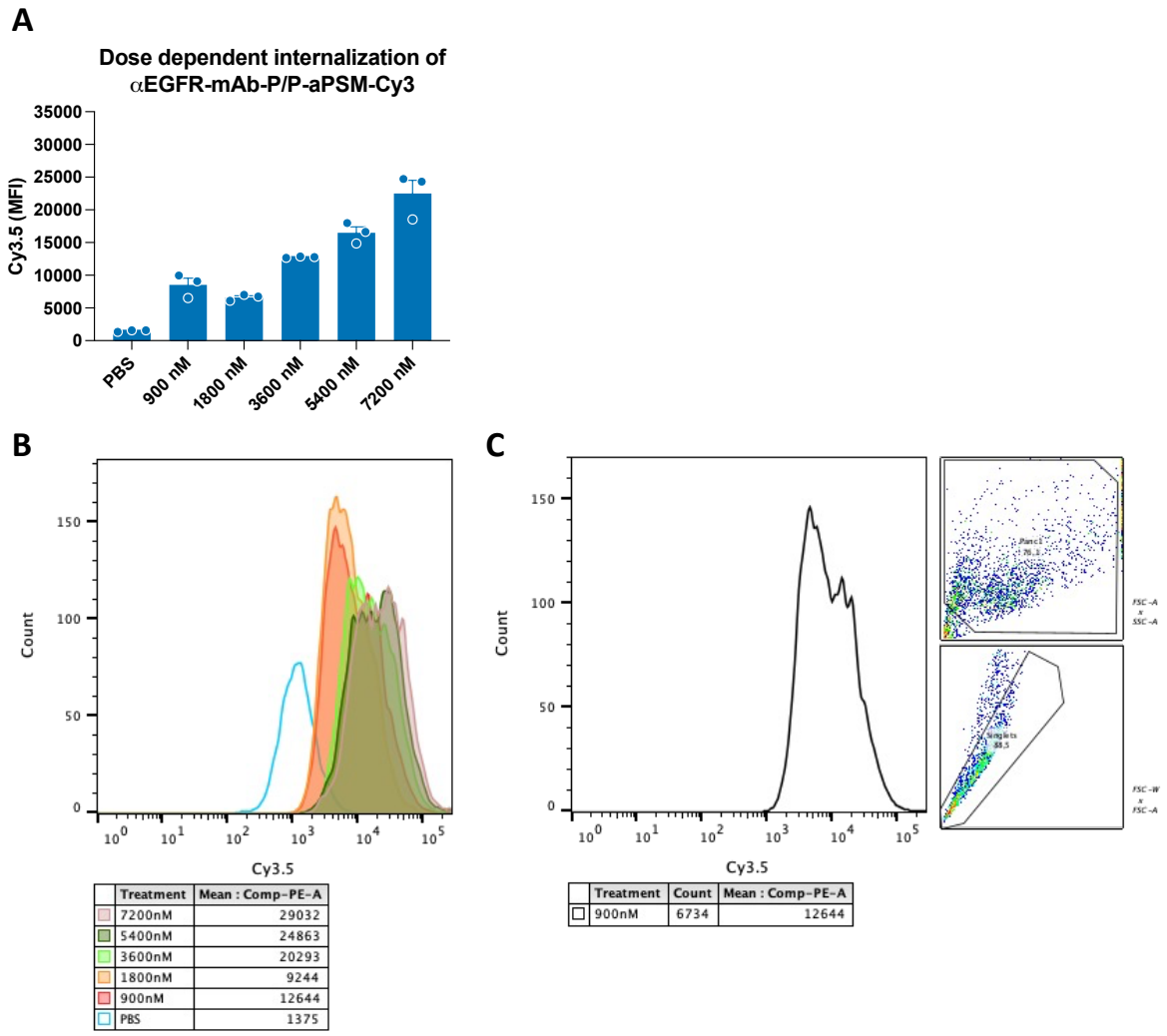


Figure S11: Flow cytometric analysis of αEGFR-mAb-P/P-aPSM-Cy3.5 internalization. Panc1 cells were pre-treated with αEGFR-mAb-P/P complexed with aPSM-Cy3.5 at increasing doses and analyzed via flow cytometry. Mean fluorescence intensity (MFI) was measured in PE-A chanel for Cy3.5. **A:** Cumulative data of 3 experiments. Data are presented as means ± SEM. **B:** Exemplary histogram overlay with MFI data of one experiment. **C:** Gating strategy.

Reactive oxygen species detection assay

Cell-based chemical detection of ROS-production *in vitro* was performed using the ROS-Glo H_2O_2 Assay (Promega, cat. no. G8820) according to the manufacturer's instructions. Briefly, $1x10^4$ Panc1 or A549 cells were seeded into a flat clear-bottomed 96-well plate in 80 µl medium and were treated overnight with αEGFR-mAb-P/P+aPSM-Cy3.5 nanocarrier or free aPSM-Cy3.5 at concentrations as indicated and cultivated at 37 °C and 5% CO_2. H_2O_2 substrate solution and ROS-detection solution were prepared as described by the manufacturer. Following treatment, 20 µl of H_2O_2 substrate solution was added to each well, followed by 100µl of ROS-detection solution. Luminescence was measured once using a Victor X3 Multi-Mode microplate reader (PerkinElmer) prior to illumination (timepoint: 0 minutes) and again following illumination of the appropriate wells at indicated timepoints. Dark controls were protected from illumination and measured simultaneously in the same well plate.

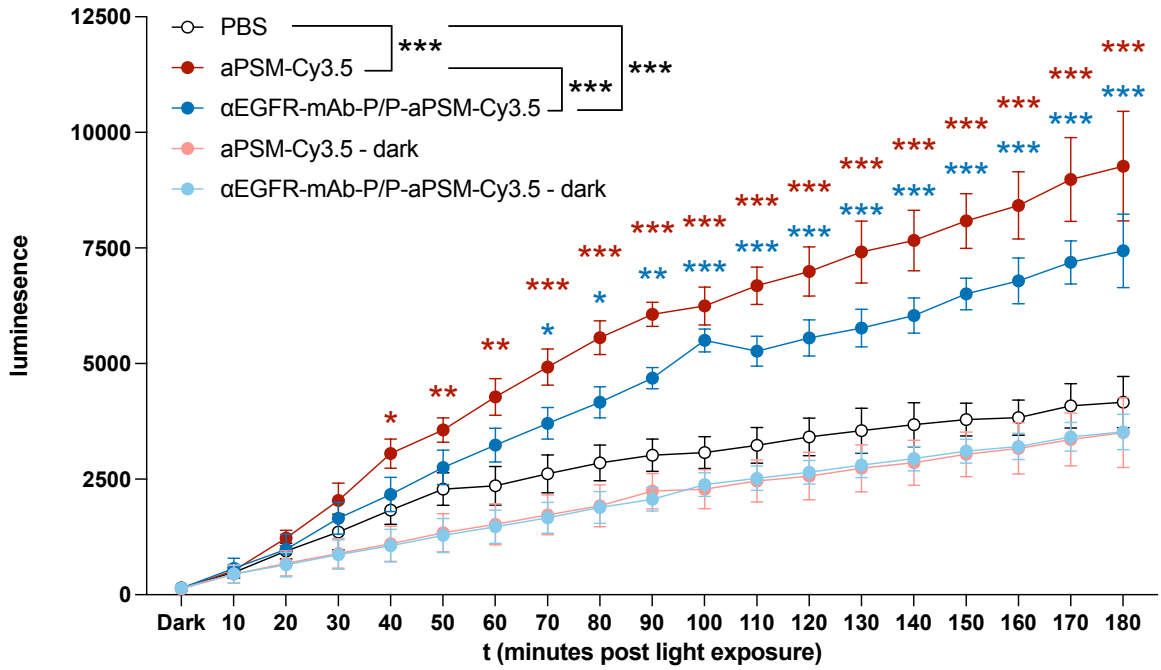
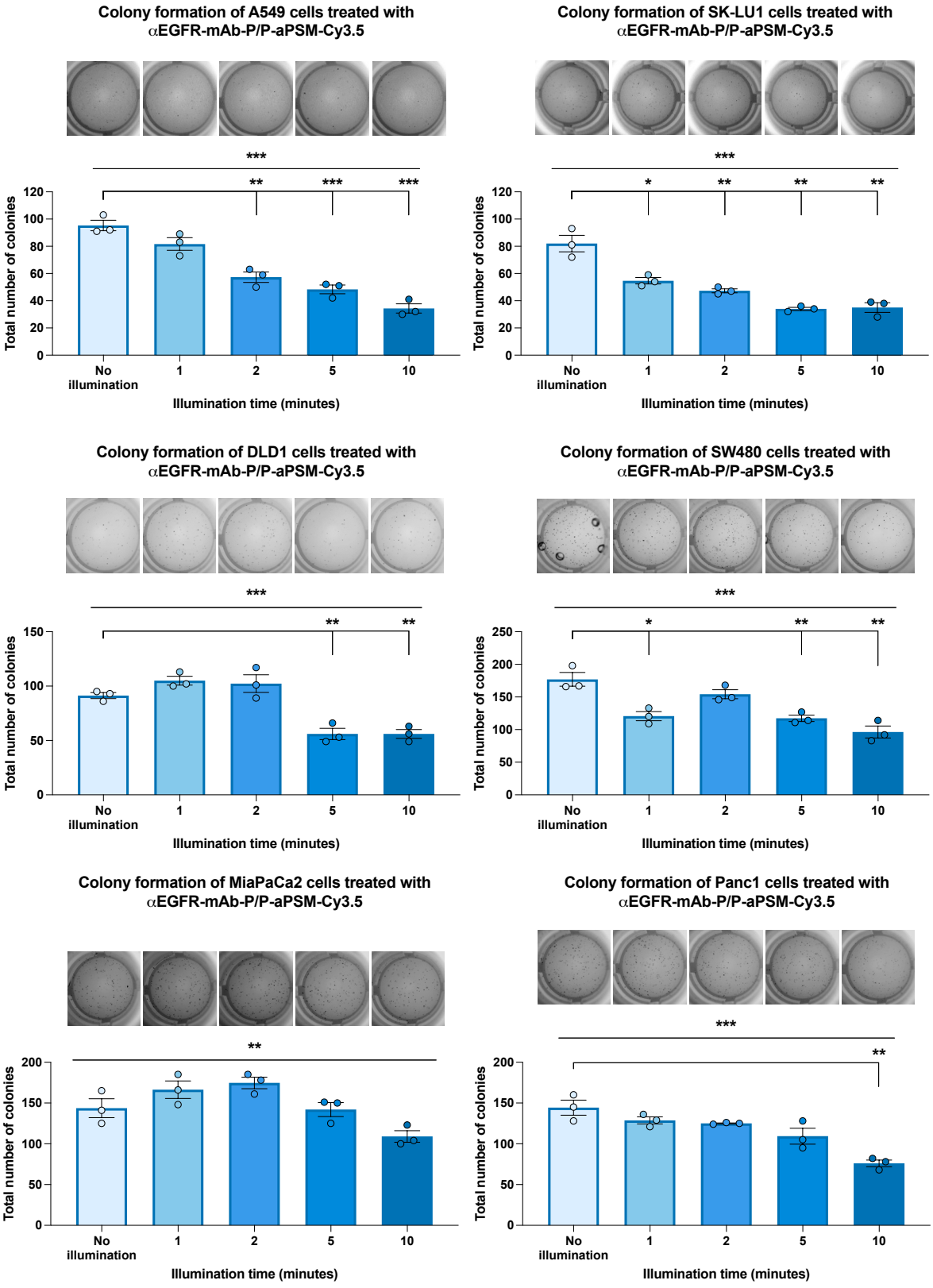


Figure S12: ROS detection assay of A549 cells pre-treated either with αEGFR-mAb-P/P (180 nM) electrostatically complexed with aPSM-Cy3.5 (5400 nM) or free aPSM-Cy3.5 (5400 nM) compound, and light-activated for the indicated time frames in minutes. Dark = no illumination. Data are presented as means ± SEM. Pair-wise comparisons was performed using two-sample t-test for independent samples (two-sided) between PBS and aPSM-Cy3.5 (red asterisk) or αEGFR-mAb-P/P+aPSM-Cy3.5 (blue asterisk) at individual timepoints. Group comparisons (black asterisk) were performed using two-way ANOVA. (*P < 0.05, **P < 0.01, and ***P < 0.001).

Colony forming assays

Colony formation assay was performed as previously described.[5,6,7,8,9] Two hundred cells of each cell line were treated with the indicated αEGFR-mAb-P/P+aPSM-Cy3.5 nanocarrier for 2 h at 37 °C and 5% CO_2, and subsequently illuminated with a red-light source for indicated times. Subsequently, the cells were seeded in soft agar (Difco Agar Noble) in 96-well plates and incubated for 5–7 days at 37 °C and 5% CO_2. The assay was then stained with 1 mg/ml INT (iodonitrotetrazolium chloride), incubated over night at 37 °C and colonies were counted using a binocular microscope.

A


Colony formation of A549 cells treated with αEGFR-mAb-P/P-aPSM-Cy3.5
Total number of colonies
Illumination time (minutes)
No illumination
1
2
5
10
Colony formation of SK-LU1 cells treated with αEGFR-mAb-P/P-aPSM-Cy3.5
Total number of colonies
Illumination time (minutes)
Colony formation of DLD1 cells treated with αEGFR-mAb-P/P-aPSM-Cy3.5
Total number of colonies
Illumination time (minutes)
Colony formation of SW480 cells treated with αEGFR-mAb-P/P-aPSM-Cy3.5
Total number of colonies
Illumination time (minutes)
Colony formation of MiaPaCa2 cells treated with αEGFR-mAb-P/P-aPSM-Cy3.5
Total number of colonies
Illumination time (minutes)
Colony formation of Panc1 cells treated with αEGFR-mAb-P/P-aPSM-Cy3.5
Total number of colonies
Illumination time (minutes)

B

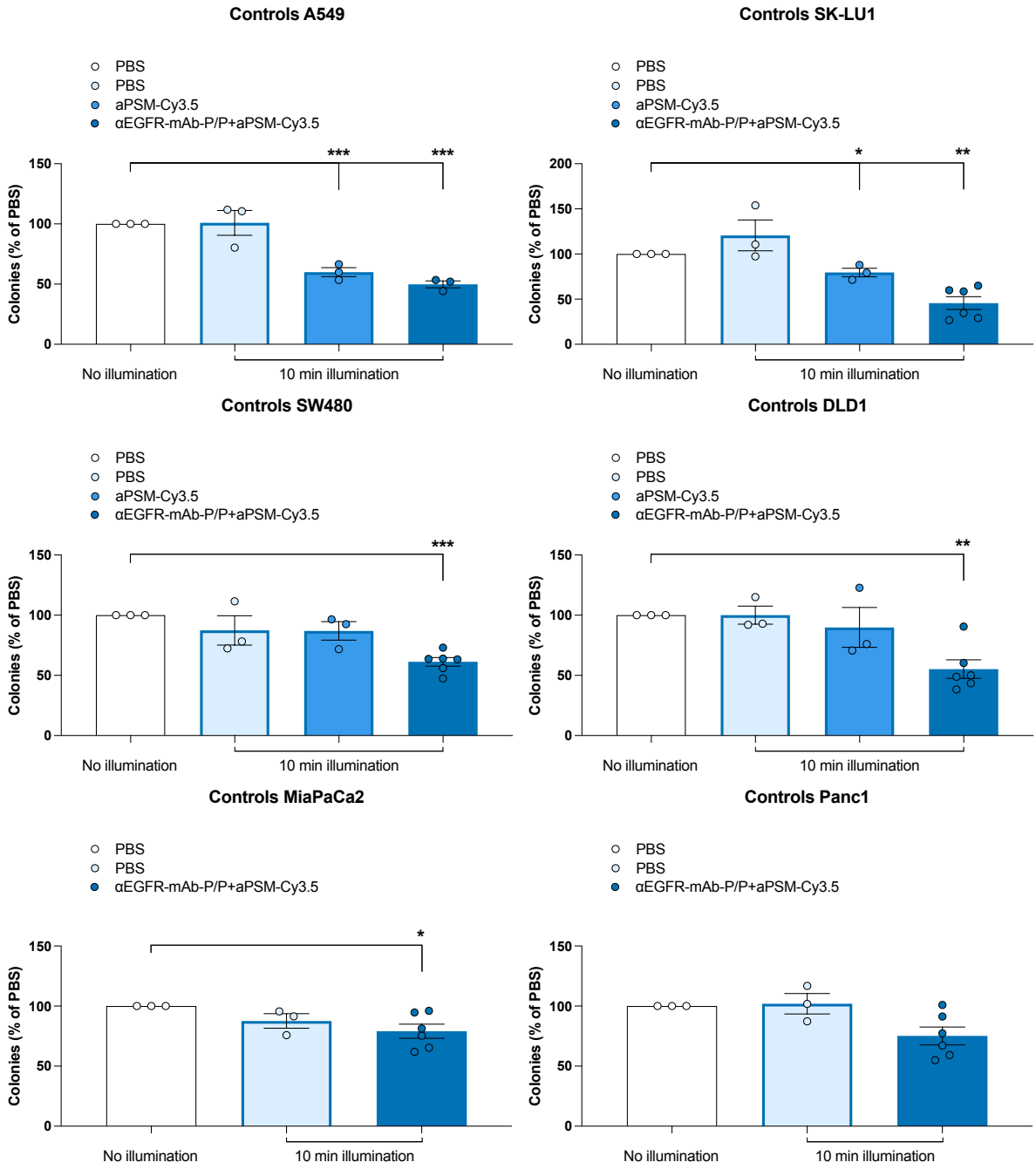


Figure S13:

A: Colony formation of A549, SK-LU1, SW480, DLD1, MiaPaCa2 and Panc1 cells pre-treated with αEGFR-mAb-P/P (60 nM) electrostatically complexed with aPSM-Cy3.5 (1800 nM) and light-activated for the indicated time frames in minute

B: Illumination-toxicity control. Colony formation of control samples for A549, SK-LU1, SW480, DLD1, MiaPaCa2 and Panc1 cells pre-treated with PBS ± 10 minutes illumination or aPSM-Cy3.5 (1800nM) with 10 minutes illumination, in comparison to treatment with αEGFR-mAb-P/P (60 nM) complexed with aPSM-Cy3.5 (1800 nM) with 10 minutes illumination. Data are presented as means ± SEM. Pair-wise comparisons was performed using two-sample t-test for independent samples (two-sided). Group comparisons were performed using regular one-way ANOVA. (*P < 0.05, **P < 0.01, and ***P < 0.001)

6. Statistical analysis

Data are presented as means ± standard error mean if not indicated otherwise. All p-values comparing two groups are representing pair-wise comparisons using the two-sample t-test for independent samples (two-sided). All p-values comparing multiple groups are presenting either a regular one-way or two-way analysis of variance (ANOVA). P-values <0.05 were accepted as indicating significant differences (*P < 0.05, **P < 0.01, and ***P < 0.001). GraphPad Prism Software Version 10.4.1 (532) was used to calculate and plot the data.

7. NMR and MS Spectra

7.1. *d,l*-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)phthalonitrile (**1**)

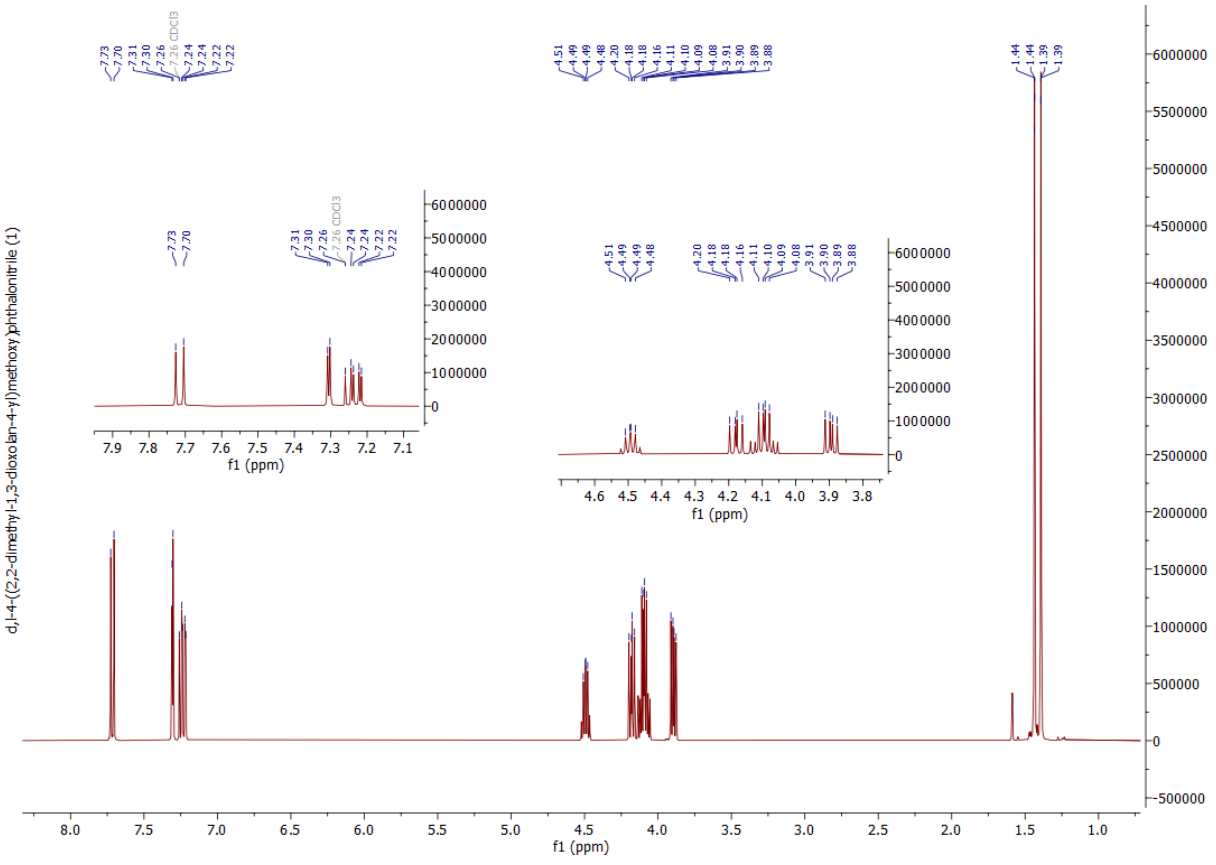


Fig. S14. ^{1}H-NMR spectrum (400MHz, $CDCl_3$) of *d,l*-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)phthalonitrile (**1**).

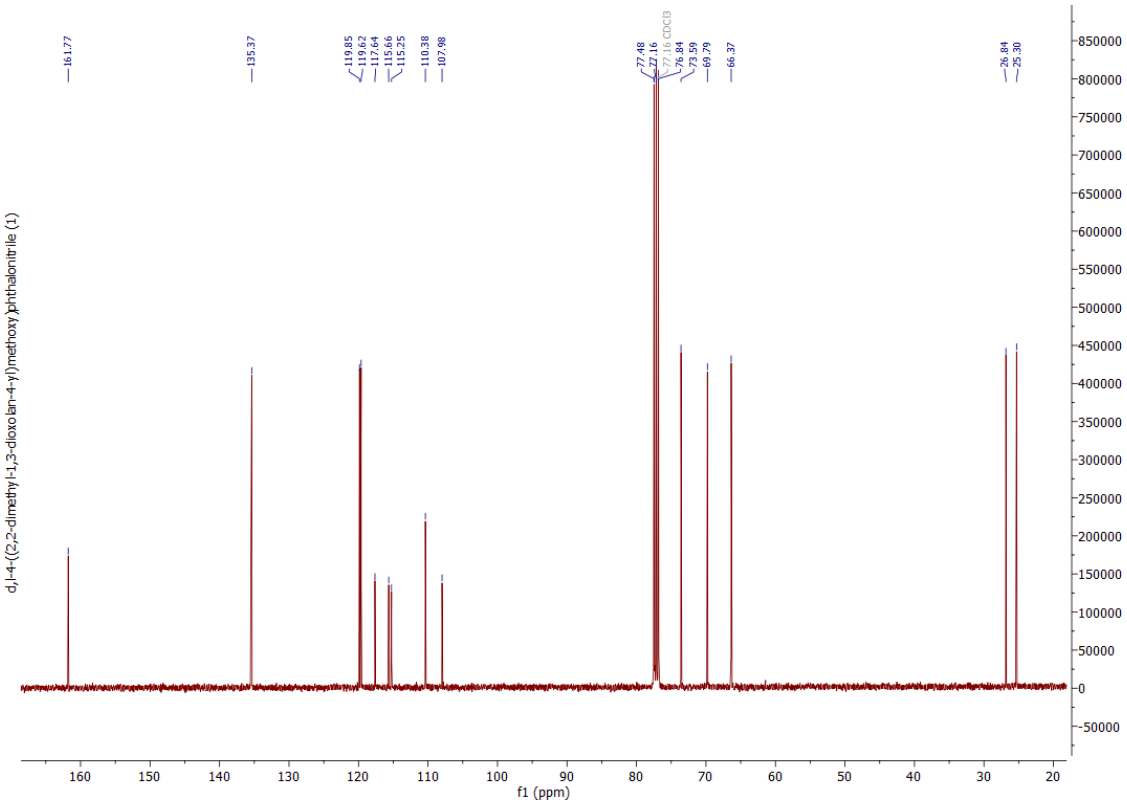


Figure S15. $^{13}C\{^{1}H\}$-NMR spectrum (101MHz, $CDCl_3$) of *d,l*-4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)phthalonitrile (**1**).

7.2. 4-[12-Hydroxy-(1,4,7,10-tetraoxadodecyl)] phthalonitrile (**2**)

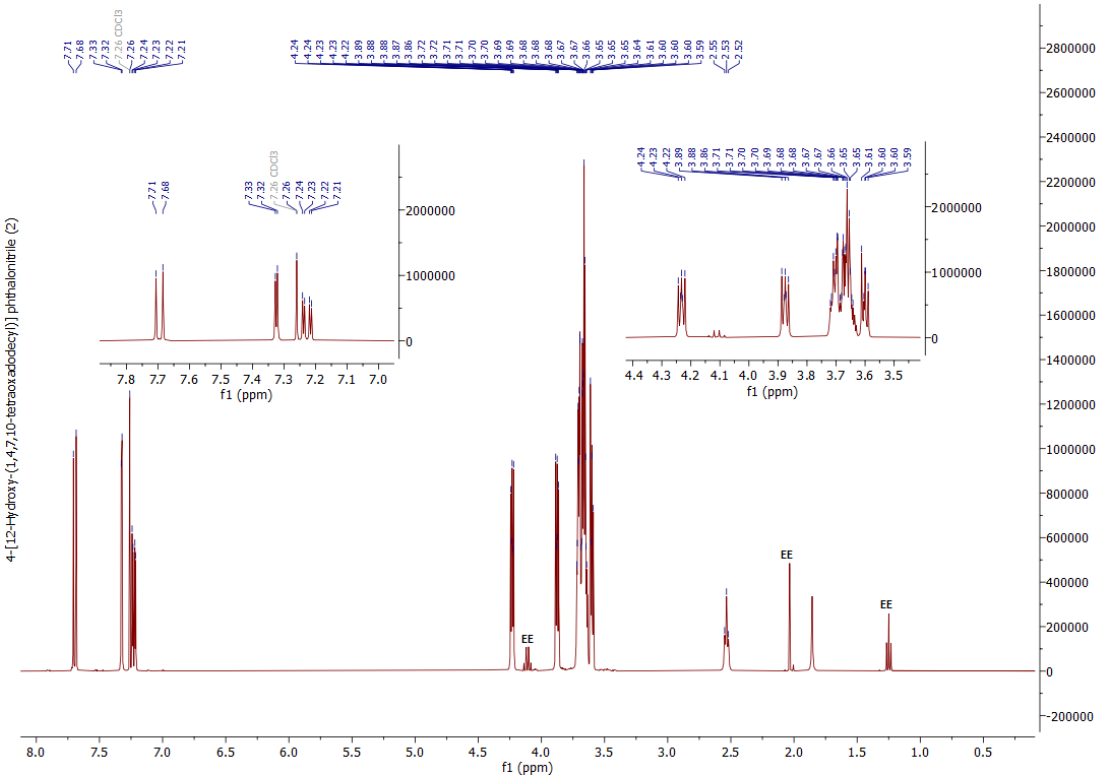


Figure S16. ^{1}H-NMR spectrum (400MHz, $CDCl_3$) of 4-[12-Hydroxy-(1,4,7,10-tetraoxadodecyl)] phthalonitrile (**2**).

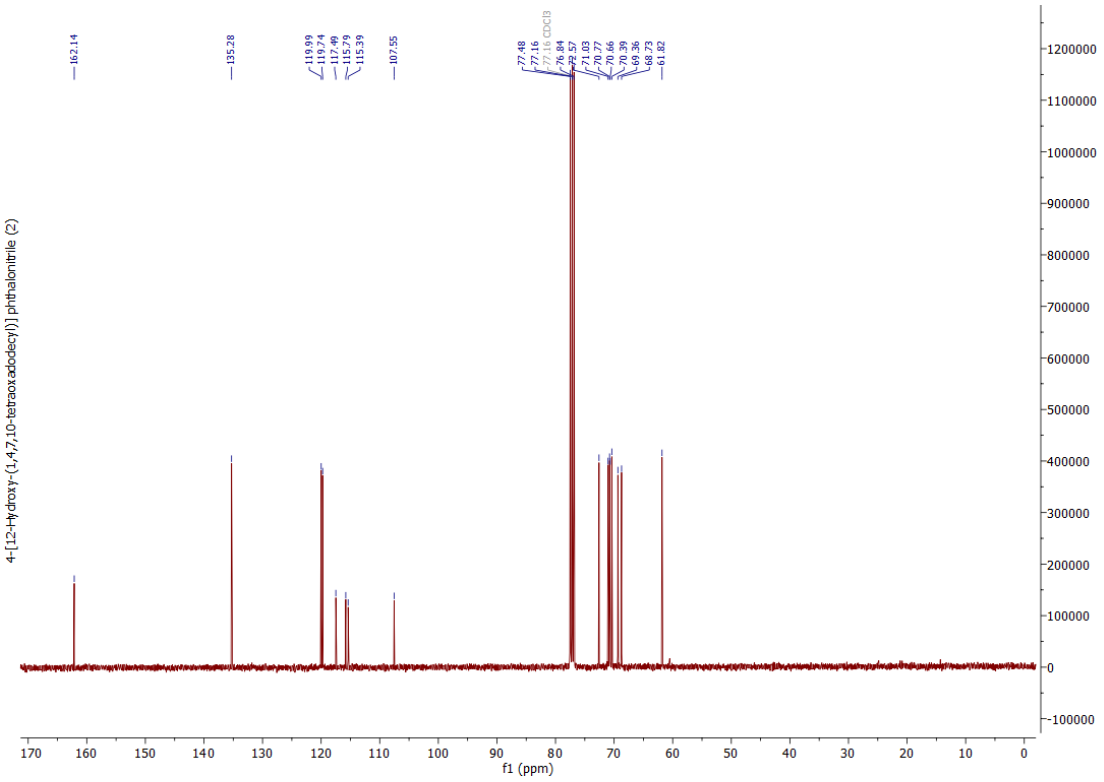


Figure S17. $^{13}C\{^{1}H\}$-NMR spectrum (101MHz, $CDCl_3$) of 4-[12-Hydroxy-(1,4,7,10-tetraoxadodecyl)] phthalonitrile (**2**).

7.3. [2(3),9(10),16(17)-tri-(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy-23-(12-hydroxy-1,4,7,10-tetraoxadodecyl)]phthalocyaninato Zn(II) (**3**)

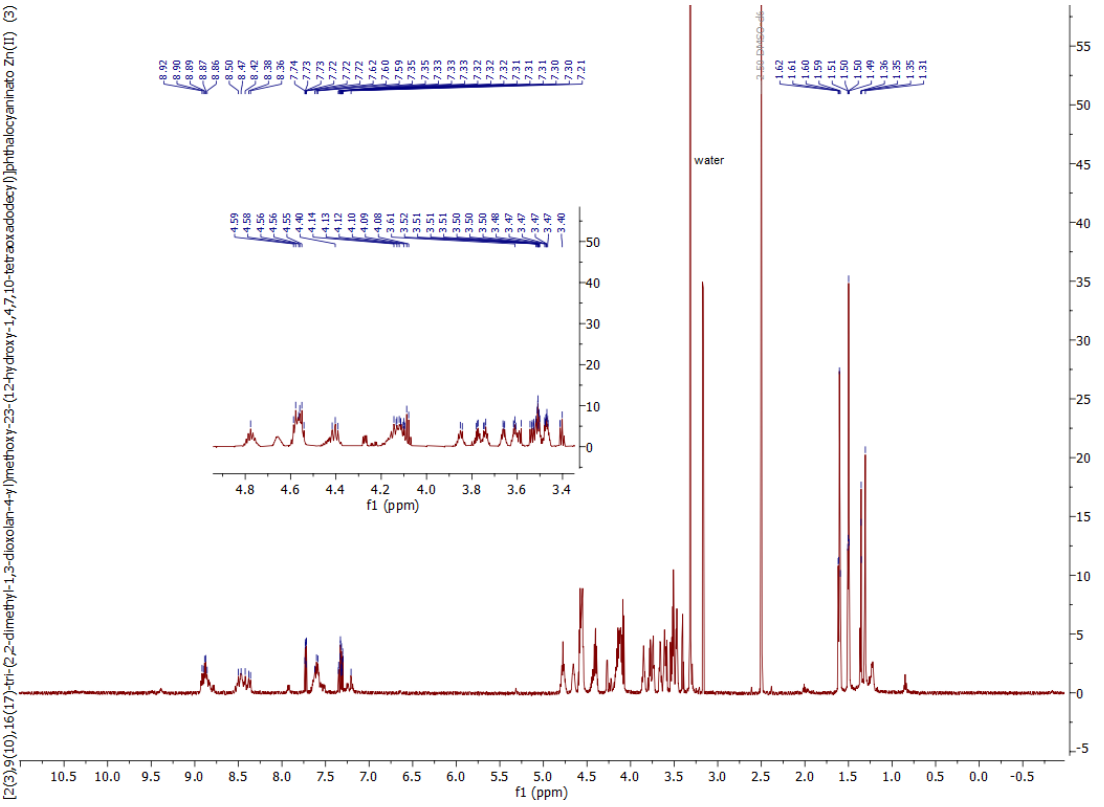


Figure S18. ^{1}H-NMR spectrum (600MHz, d_6-DMSO) of [2(3),9(10),16(17)-tri-(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy-23-(12-hydroxy-1,4,7,10-tetraoxadodecyl)]phthalocyaninato Zn(II) (**3**).

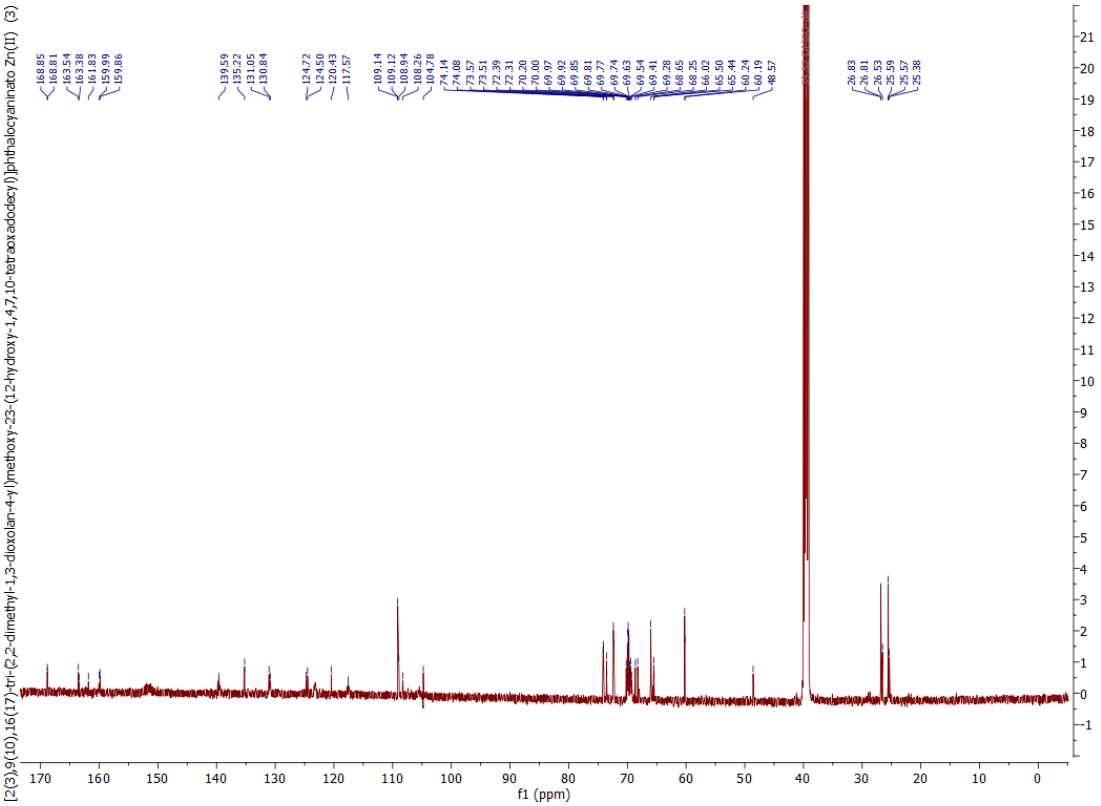


Figure S19. ^{13}C-NMR spectrum (151MHz, d_6-DMSO) of [2(3),9(10),16(17)-tri-(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy-23-(12-hydroxy-1,4,7,10-tetraoxadodecyl)]phthalocyaninato Zn(II) (**3**).

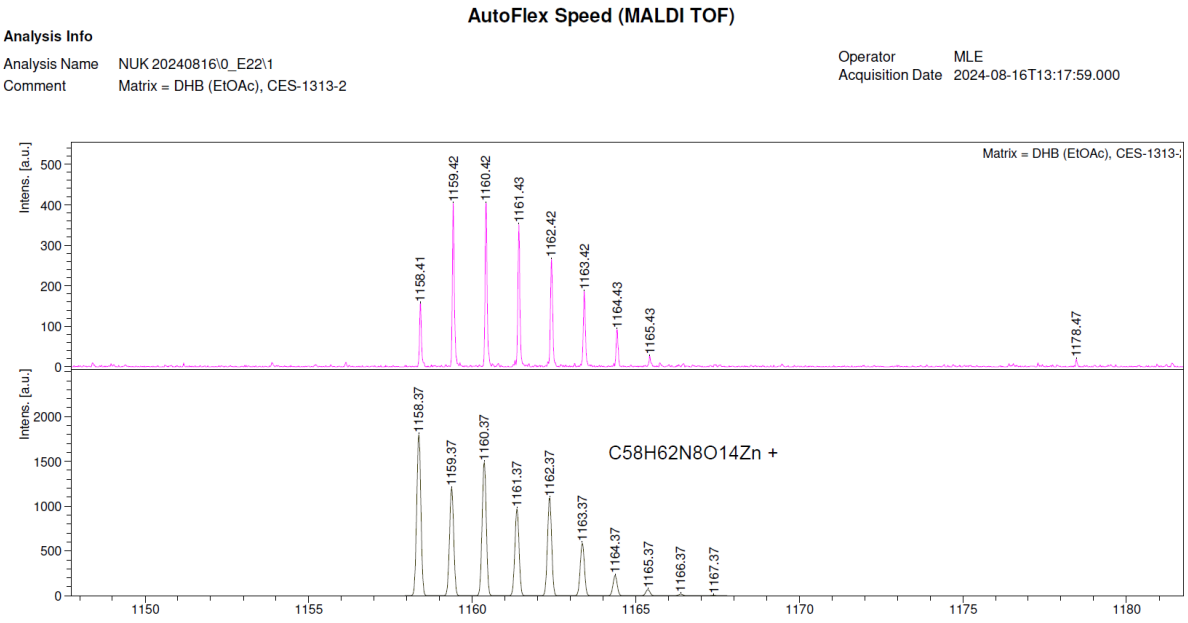


Figure S20. Mass spectrum (MALDI-ToF (DHB, EtOAc)) of [2(3),9(10),16(17)-tri-(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy-23-(12-hydroxy-1,4,7,10-tetraoxadodecyl)]phthalocyaninato Zn(II) (**3**).

7.4. [2(3),9(10),16(17)-tri-(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy-23-(12-methylsulfonyloxy-1,4,7,10-tetraoxadodecyl)]phthalocyaninato Zn(II) (**4**)

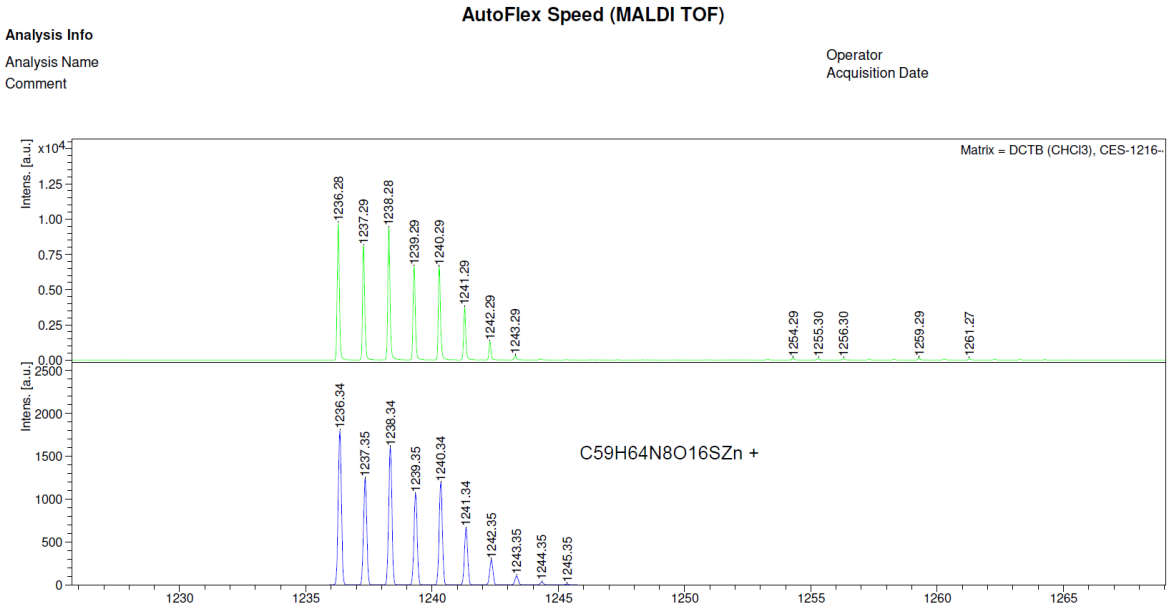


Figure S21. Mass spectrum (MALDI-ToF (DHB, $CHCl_3$)) of [2(3),9(10),16(17)-tri-(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy-23-(12-methylsulfonyloxy-1,4,7,10-tetraoxadodecyl)]phthalocyaninato Zn(II) (4).

7.5. [2(3),9(10),16(17)-tri-(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy-23-(12-azido-1,4,7,10-tetraoxadodecyl)]phthalocyaninato Zn(II) (**5**)

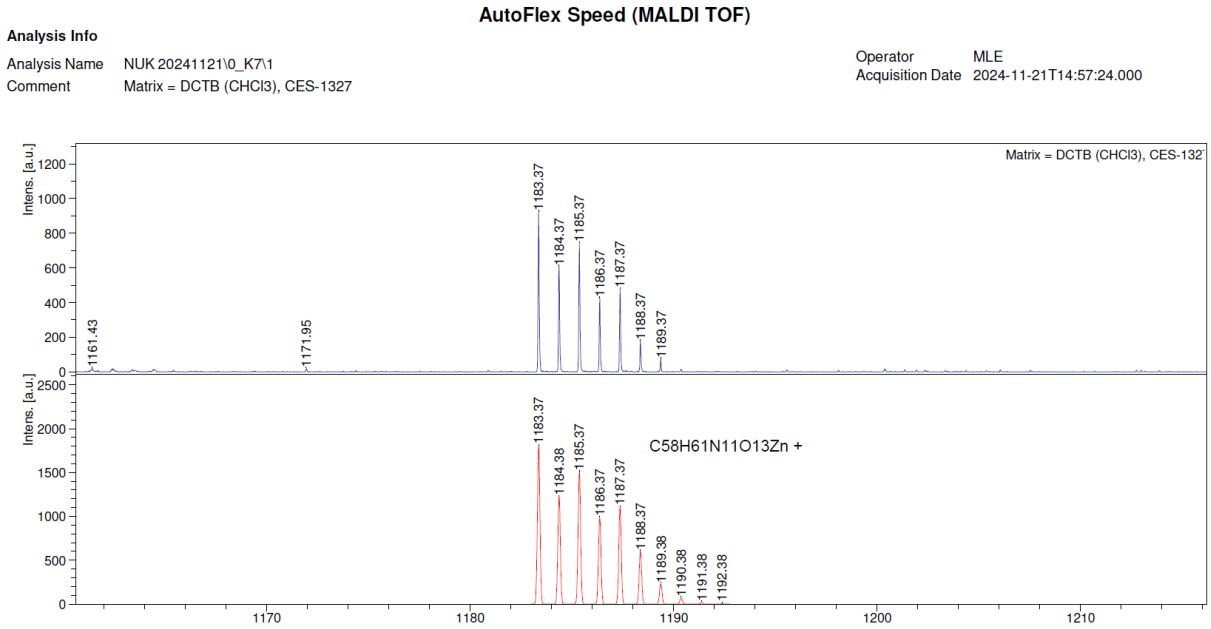


Figure S22. Mass spectrum (MALDI-ToF (DHB, $CHCl_3$)) of [2(3),9(10),16(17)-tri-(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy-23-(12-azido-1,4,7,10-tetraoxadodecyl)]phthalocyaninato Zn(II) (**5**).

7.6. aPSM-Cy3.5 (**6**)

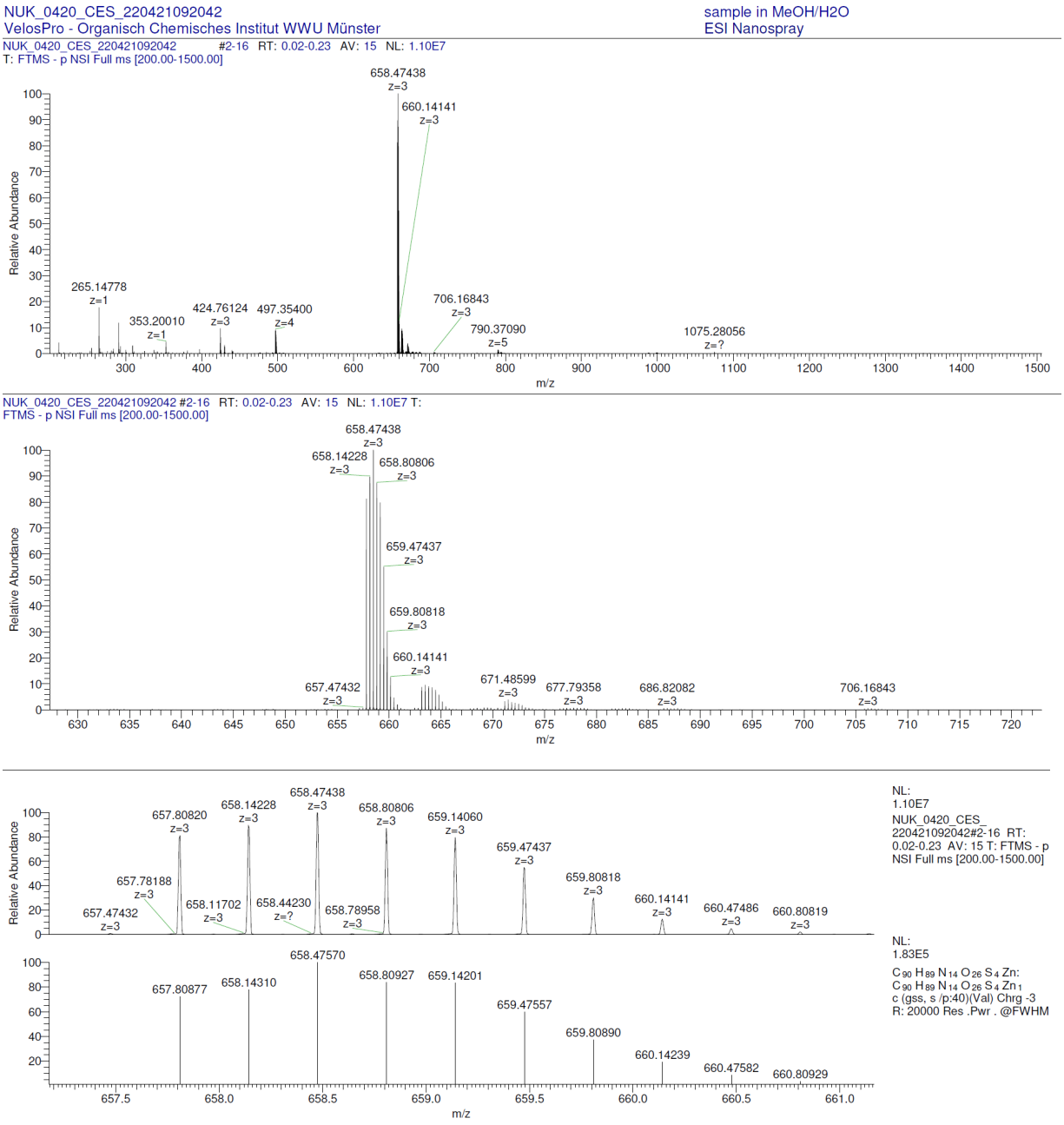
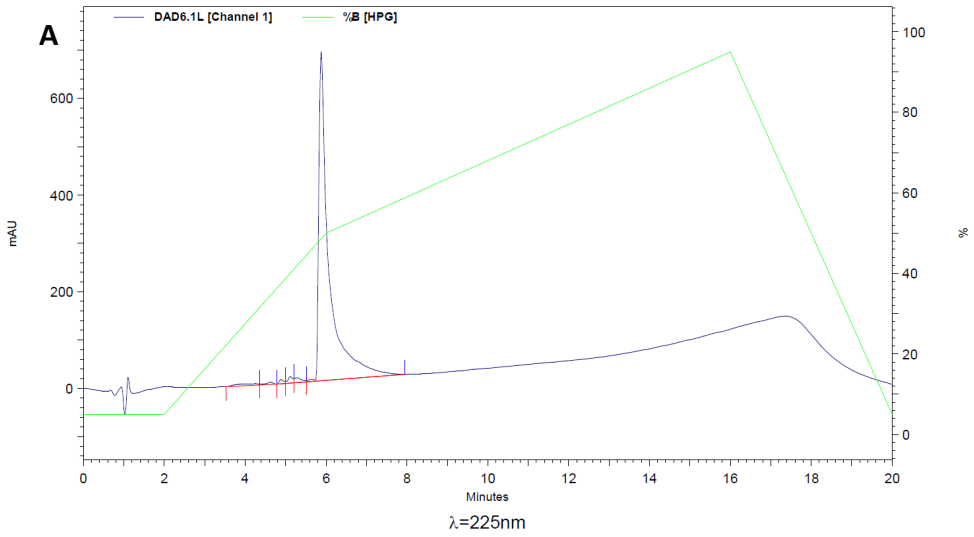


Figure S23. Mass spectrum (HRMS (ESI-)) of aPSM-Cy3.5 (**6**).

A

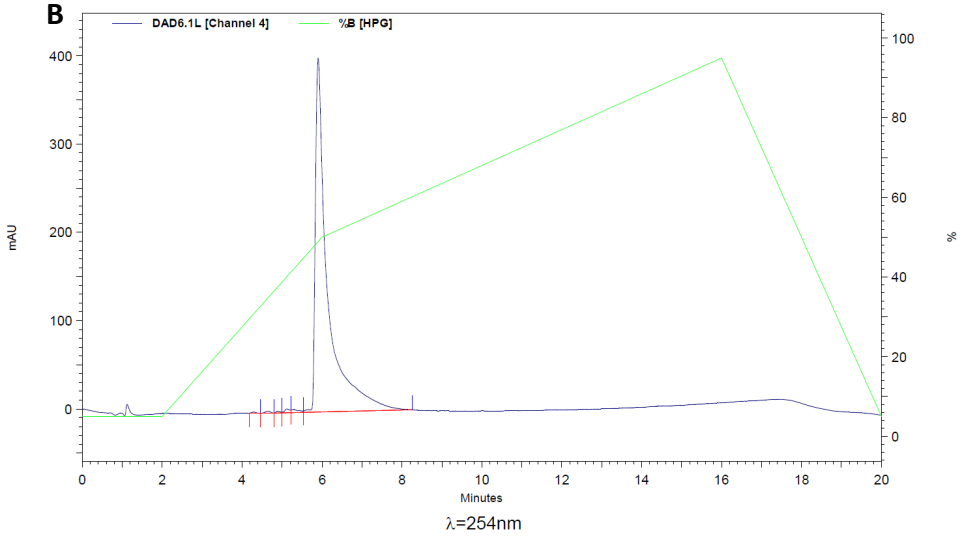


λ=225nm

DAD6.1L [Channel 1]
Results

Retention Time	Area	Area %
4,267	131369	1,06
4,617	59603	0,48
4,883	67313	0,54
5,117	108419	0,87
5,283	125546	1,01
5,883	11945403	96,04
Totals	12437653	100,00

B

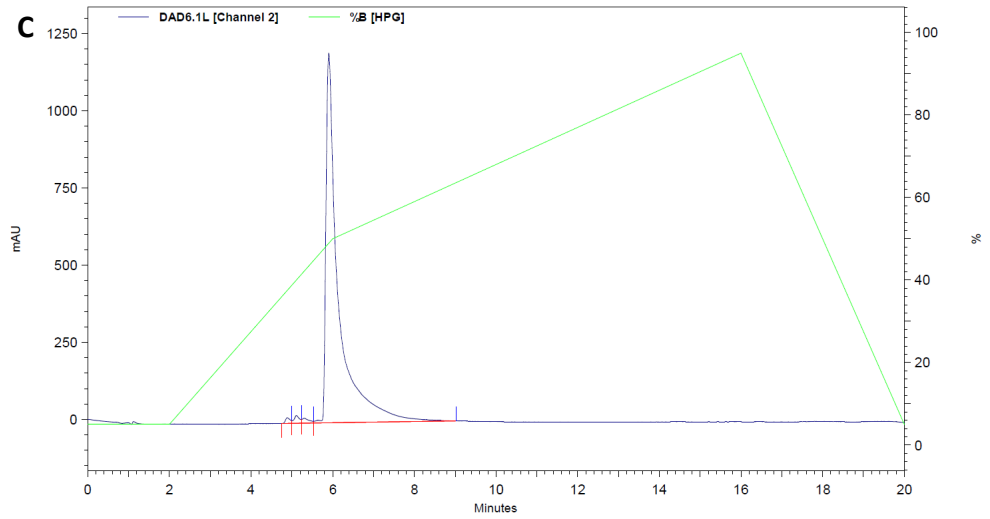


λ=254nm

DAD6.1L [Channel 4]
Results

Retention Time	Area	Area %
4,283	10181	0,12
4,650	20412	0,24
4,900	15120	0,18
5,117	37136	0,43
5,300	46191	0,54
5,900	8497335	98,50
Totals	8626375	100,00

C

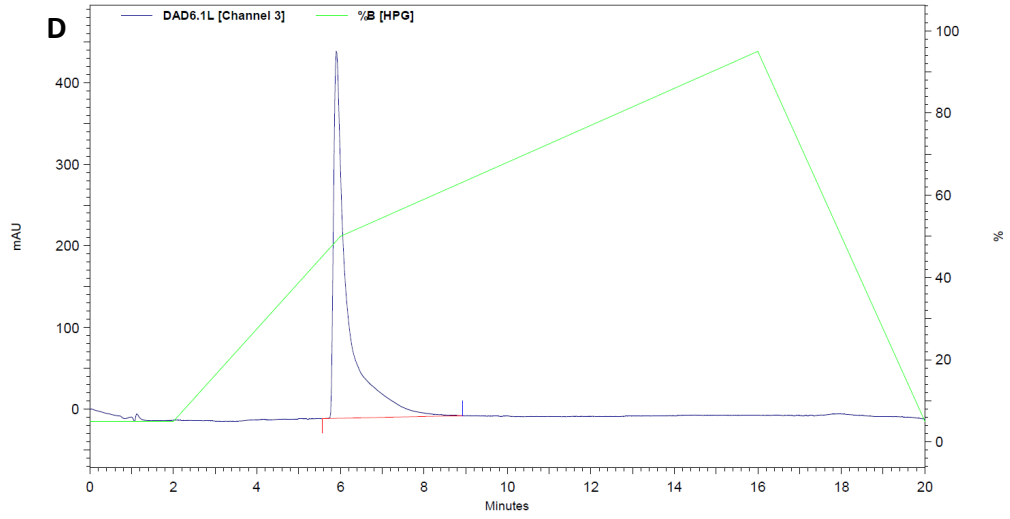


λ=580nm

DAD6.1L [Channel 2]
Results

Retention Time	Area	Area %
4,883	144678	0,56
5,117	226277	0,87
5,300	191268	0,74
5,900	25312404	97,83
Totals	25874627	100,00

D



λ=675nm

DAD6.1L [Channel 3]
Results

Retention Time	Area	Area %
5,900	10078122	100,00
Totals	10078122	100,00

Figure S24. Analytical HPLC of aPSM-Cy3.5 (**6**) (quality control) at different wave lengths (225nm (**A**), 254nm (**B**), 580nm (**C**), 675nm (**D**)).